Proposal/Contract no.: **INCO-2006-CT-032296**
Project acronym: **SavinMucoPath**
Project full title: **Novel Therapeutic and Prophylactic Strategies to Control Mucosal Infections by South American bacterial strains**

FINAL ACTIVITY REPORT
from October 2006 to September 2009

Period covered from :	**01-10-2006 to 30-09-2009**
Start date of project :	**01-10-2006**
Duration :	**3 years (30-09-2009)**
Project coordination name :	**Jean Claude SIRARD**
Project coordinator organisation name :	**INSERM**

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1. Publishable Executive Summary

1.1. OBJECTIVES

Among the communicable diseases that affect the public health in developing countries there are infectious and respiratory diseases, diarrhoea and food-borne diseases. We have chosen to study bacterial pathogens that cause such diseases and have a profound impact on health in South America, namely *Streptococcus pneumoniae*, *Salmonella enterica* serovar Enteritidis, and *Bordetella pertussis.* A common feature for these diseases is that they are caused by pathogens entering by and colonizing mucosa. Therefore, understanding host-pathogen interactions at the mucosal sites is essential for development of effective immuno-intervention measures.

The specific scientific objectives were to:

1. Characterize the mucosal innate response to the pathogens. As simultaneous mucosal infections are the norm, the influence of co-infections on this response was also analyzed. *We succeeded in this task.*
2. Identify pathogen-associated molecular patterns that stimulate natural mucosal immune defenses; these molecules could be used as adjuvants for vaccine formulation or as therapeutic agent to enhance the protective mucosal responses. *We made the proof of concept with flagellin but we did identify novel molecules. This failure is mainly due to the delay in obtaining our reporter animal models.*
3. Evaluate the efficacy of innovative approaches that enhance pathogen-specific response or prevent excessive inflammatory response. *We succeeded in this task.*

This proposal aimed also to achieve technical objectives by:

1. The delivery of selected bacterial compounds including scaling-up processes in order to evaluate their potential use as vaccine components in large-scale production.
2. The development of screening assays to detect bacterial compounds that induce mucosal immunity for further therapeutic and vaccine studies.
3. The generation of site-directed bacterial mutants for analysis of *Salmonella* virulence and contribution of PAMP in mucosal immunity.

Our general aim was to provide basic knowledge that will allow the development of novel mucosa-specific therapeutics and vaccines to control infections by *S. pneumoniae*, *S. enterica*, and *B. pertussis*. *In the consortium, we made significant findings that will support such development.*

1.2. CONTRACTORS INVOLVED

Contractor Role*	Partner or Contractor n°	Participant n°	Participant name	Contractor short name	Country
CR/CO	1	1	Institut National de la Santé et de la Recherche médicale	INSERM	France
CR**	2	2	Universidad Nacional de la Plata	UNLP	Argentina
CR**	2	3	Universidad Nacional de la Plata	UNLP	Argentina
CR	3	4	Imperial College London	IMPCOL	United Kingdom
CR	4	5	Swiss Federal Institute of Technology (ETH) Zurich	ETHZ	Switzerland
CR	5	6	Universidad de la Republica Oriental del Uruguay	UDELAR	Uruguay
CR	6	7	Pontificia Universidad Catolica de Chile	PUCC	Chile
CR	7	8	Instituto Biologico Argentino	BIOL	Argentina

*CO = Coordinator; CR = Contractor
** there are 2 participants that are represented legally by the same contractor: UNLP (Participant 2 is the research group directed by Dr. Martin Rumbo and participant 3 is the one directed by Prof. Daniela Hozbor)

1	2	3	4
Dr Jean Claude Sirard	Dr Martin Rumbo	Dr Daniela Hozbor	Dr Tracy Hussell
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5	6	7	8
Dr Wolf-Dietrich Hardt	Dr Jose Alejandro Chabalgoity	Dr Alexis Kalergis	Dr Augusto Pich Otero
Institute of Microbiology, Department of biology, Zürich, Switzerland	Department of Biotechnology, School of medicine, Institut of Hygiene, Laboratory for Vaccine Research, Montevideo, Uruguay	Department of Molecular Genetics and Microbiology, Laboratory of MicrobiologySantiago Chile	Instituto Biologico Argentino Buenos Aires Argentina

1.3. COORDINATION CONTACT DETAILS :

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1.4. WORK PERFORMED AND RESULTS ACHIEVED

We have selected isolates specific for Latin American countries including *Streptococcus pneumoniae* serotype 1, *Salmonella enterica* serovar Enteritidis, and *Bordetella pertussis.* These pathogens are locally associated with important rates of morbidity and mortality. For *S.* Enteritidis, we also used the phylogenetically close strain: Salmonella *enterica* serovar Typhimurium. ***The specific objectives achieved are:***

a. The delivery or the development of infection and co-infection models in laboratory animals (mice) by mucosal route.
b. The transcriptional analysis of mucosal protective responses.
c. The analysis of innate protective mechanisms in infections with *Bordetella*, *Streptococcus* and *Salmonella.*
d. The generation of animal models for screening bacterial compounds that induce mucosal innate and adaptive immunity (*CCL20-luc, CXCL1-luc,* and *CXCL2-luc*).
e. The dissection of protective mechanisms and pathogenic processes altering innate responses for *Salmonella.*
f. The proof of concept that a pathogen-associated molecular pattern (PAMP) can be used for therapeutic and prophylactic purposes
g. The analysis of how pre-existing challenge can modify the outcome of therapeutic or prophylactic treatments in lungs.

Our major results can be summarized as follows:

1. We showed that flagellin is a potent molecule for innate of clearance of mucosal infections.
2. We found that flagellin-mediated mucosal adjuvant effect mainly requires epithelial signaling. These findings will be instrumental to develop new therapeutic and vaccinal approaches for mucosal infections.
3. We found that outer membrane vesicles and TLR4 agonists are appropriate candidates for intranasal anti-pertussis vaccine.
4. We established the major role of TLR4 signaling in the early protection against pertussis.
5. We developed novel transgenic mice to screen immunomodulatory molecules derived from pathogens.
6. We characterized human intestinal mucosa cells and associated lymphoid structures.
7. We have identified a post-inflammation refractory phenotype in the lung following inflammation that pre-disposes to bacterial super-infection.
8. We have further identified a major role for the negative regulator CD200R in innate immune homeostasis in the respiratory tract.
9. We identified that blockade of OX40, a T cell co-stimulatory receptor reduce lung inflammatory disease. To prevent infection in the first place, we propose to tailor immune receptors to act as "smart adjuvants" to enhance anti-pathogen immunity.

10. We observed that protective response against *Salmonella* colitis is mediated by LPS-specific antibodies. This might represent the basis for the future design of a vaccine against *Salmonella* diarrhea.
11. We identified a key role of mucosal dendritic cells and Caspase-1 activation in enterocytes in acute *Salmonella* enterocolitis.
12. We identified the key pathways and cytokines induced by *Salmonella* in the infected gut mucosa. Their targeting by appropriate drugs may represent a novel strategy to prevent inflammatory colitis.
13. We delineated a role for Th17 responses in protective immunity against *S. pneumoniae*
14. We demonstrated that it is possible to prepare an effective intranasal whole cell vaccine against the most relevant *S. pneumonia*e strains circulating in South America
15. We showed that *S.* Typhimurium impairs antigen presentation by DCs to T cells, due to virulence factors in pathogenicity islands 1 and 2. This process is host specific, and restricted to the DCs derived from host in which Salmonella causes systemic infection.
16. We developed a high yield Salmonella flagellin production process.
17. We found that bacterial extract used in a pharmaceutical product (INESPECIN) activate innate immunity.
18. We produce *B. pertussis* culture in large scale to isolate new antigens.

1.5. EXPECTED END RESULTS AND INTENTION FOR USE AND IMPACT

We intend to define new intervention strategies to cure or prevent diseases triggered by the bacterial isolates specific for Latin American countries.

- **Our Project enhanced significantly the general knowledge on mucosal pathogens and mucosal immunity. Our findings open prospects for development of new therapies and vaccines to manipulate the innate and adaptive immunity. Efforts will be done to go one step further than the proof of concept in the development.**
- **Our project enabled to construct the collaborative network to study the targeted pathogens and to improve the competitiveness of all partners.**
- **Anti-pertussis vaccine development for Argentinean society has been initiated by partner 2. This effort was supported by our consortium.**
- **Our consortium trained several high qualified personel and student. Among them, 8 persons have got a permanent position within the consortium teams or elsewhere.**
- **Most data generated in our consortium have been published and are thus accessible to the scientific community and industries.**

1.6. MAIN ELEMENTS FOR PUBLISHABLE RESULTS OF PLAN FOR USING AND DISSEMINATING THE KNOWLEDGE.

Thirty four articles and review articles have been published during the 3-years period of activity. We plan another 8-10 articles within the next year (see last section). In addition, national and international conferences have been attended by all partners. Similarly, we will continue to communicate the results achieved within the consortium to meetings in 2010 and later on.

We have organized in 2007 in Argentina (at La Plata) a course of “Mucosal Innate and Adaptive Immunity” involving most PIs from our consortium as teachers. This course targeted more than 50 Latin American PhD students and post-doctoral fellows. In 2008, we organized in Uruguay (Montevideo) a course of “Transcriptomic analysis of immune responses to pathogens” involving most PIs, PhD students and post-doctoral fellows from our consortium.

Actual dates	Type	Type of audience	Countries addressed	Size of audience
2006	1 web-site dedicated to the project	Research and public	Any	Large
2006-2009	34 articles	Research	Any	Large
2006-2009	>30 conferences	Research	National and international	From 20 to 500
2006-2009	2 Courses	Research	Latin American countries	40 and 15
2007	1 video	Research and public	Any	Large

The consortium plan the following dissemination activities with the data generated during the project:

Planned dates	Type	Type of audience	Countries addressed	Size of audience
2010	8-10 Publications	Research	Any	Large
2010	10 conferences	Research	National and international	From 50 to 200

1.7. PROJECT LOGO AND WEBSITE :

Project logo : 

Public website : http://www.lille.inserm.fr/site/savinmucopath/

2. Final Report

2.1 PROJECTS OBJECTIVES AND MAJOR ACHIEVEMENTS

2.1.1 Objectives

The strategic objective is to confront the emergency caused by mucosal pathogens– *Streptococcus pneumoniae*, *Salmonella spp,* and *Bordetella pertussis* – through the improvement of knowledge on molecular pathogenesis and the development of novel therapeutic and prophylactic interventions. The control of these neglected communicable diseases is essential to improve the health and decrease the morbidity and mortality that they cause within the Latin American population, especially in children and communities with the lowest socio-economic status. Respiratory and enteric infections are among the most prevalent cause of diseases in the childhood. The third leading cause of death worldwide is respiratory tract infections and, thereby, a World Health Organization (WHO) priority for vaccine development. **The ultimate goal of the Project is to find novel solutions to control theses neglected diseases.**

The vaccine industry has been reluctant to commit resources to the development of vaccines for some of the world's developing and poorest countries. Most vaccines developed against diseases are specific for pathogenic strains found in Europe or North America and, thereby, are poorly effective on local infections. This proposal is critical for developing new vaccines for the selected mucosal diseases. Furthermore, local research aimed to boost immunity against mucosal pathogens is required for the development of new vaccines in Argentina, Chile and Uruguay. This is a real issue because these countries totally depend on vaccines produced abroad. On the other hand, treatment with antibiotics is not really accessible to individuals of low socio-economic status, and is facing the emergence of multi-resistant strains. The production of these chemotherapies, either covered or not by patents is controlled by pharmaceutical companies and the treatments remain cost-effective approaches.

Our proposal will give a specific contribution to the identification of novel bacteria-derived immuno-stimulators / adjuvants, so-called PAMPs, specific for mucosa which should enhance locally the effectiveness of chemotherapy as well as the effectiveness of the adaptive immune responses specific for mucosal microbial antigens. Combined with the understanding of molecular mucosal pathogenesis of pathogens of main interest for public health in Latin America, this project should contribute to development of appropriate treatment of the corresponding diseases, especially in the childhood.

The Project is aimed to provide knowledge on molecular pathogenesis of mucosal diseases and to develop novel and low-cost effective immuno-intervention strategies applicable to other mucosal infections. We will develop these approaches in cell models and rodent models, to ultimately bring candidate experimental immuno-interventions to clinical trials in Europe or South America within FP7 and with involvement of SMEs.

The use of PAMPs to enhance the mucosal innate and adaptive immune response is a new concept that has started to emerge from cutting-edge evidence accrued during the past few years. This approach could dramatically improve the efficacy of chemotherapy and vaccines against mucosal infectious agents, by providing a mucosal compartmentalized immune response. This is in contrast to PAMP used by systemic route that induce a global effect in the host mimicking septic shock. SavinMucoPath anticipates that, during an on-going infection, mucosal administration of PAMP (purified PAMP preparations and/or attenuated bacterial strains) will stimulate innate responses within the whole targeted mucosa and this process will combine to the innate response in infectious foci to better eradicate the pathogenic microbes using natural effector mechanism, like production of antimicrobial peptides. Similarly, mucosal co-administration of PAMP and selected protective antigens would constitute the development of new mucosal vaccines adjuvants, based on their unique immuno-modulating properties.

The use of specific agonists and antagonists that target receptors on antigen presenting cells (APC) and T cells, CD200R and OX40 respectively, is also new concept for the control of mucosal infections and immunopathologies. We will carry out experiments to determine the proof-

of-principle that molecules activating or inhibiting function of APC or T cells could be used to cure mucosal infections or to improve their prognostic.

It is important to emphasize that the results of this effort may have a “transdisease” impact on anti-microbial treatments and vaccine strategies for different infectious diseases. If successful, the screened molecules may have an impact on the overall existing vaccines and antibiotherapy. A successful outcome of SavinMucoPath will substantially contribute to the further development of innovative and effective projects against any neglected mucosal infections in the world.

State of the art

Mucosal tissues represent the major sites of infection by pathogenic microbes and studying mucosal pathogen is therefore relevant for infection and immunity. We plan to assess novel and innovative treatments that act directly at the site of entry and colonization of pathogens. The existing strategies to treat bacterial diseases are based on antibiotic therapies and vaccines. However, these interventions have a limited effect on the diseases caused by the 3 pathogens that we have chosen. Indeed, there is no vaccine to prevent infections mediated by *S.* Enteritidis and available vaccines for *B. pertussis and S.* pneumoniae present several drawbacks when applied in Latin American countries. Strains of pathogens currently used to produce these vaccines are the most prevalent in Europe and North America but are different from the strains encountered in Latin America. In addition, there is a significant increase of antibiotic resistance among these bacterial strains thereby limiting the use of common low-cost antibiotics. A major issue about treatments is the route of administration and bio-distribution. Therapeutics delivered by systemic routes do not provide the optimal availability of drugs at mucosal sites. Most vaccines are formulated for systemic injections and do not generate appropriate levels of mucosal responses. ***The main objective of the proposal is therefore to provide novel intervention approaches that block infections within mucosa, early enough to prevent systemic dissemination as an alternative to cost-effective antibiotics and that deliver vaccines by mucosal route to trigger protective mucosal immunity. We will in particular define novel ways to specifically boost mucosal innate and/or adaptive defence mechanisms or to interfere preferentially with virulence factors.***

Mammals have evolved defence mechanisms to prevent and contain infection of highly diverse pathogens. These innate defences are constitutively expressed at mucosal sites and represent the first barrier to mucosal colonization. However, pathogenic microorganisms are equipped to breach omnipresent innate defences in order to infect the host. Most efforts of bacteria are thereby dedicated to escape early host defences by strategies such as down-regulation of cell signalling or resistance to antimicrobial compounds. The other characteristics of pathogenic bacteria compared to indigenous microorganisms are their ability to colonize epithelial surfaces of mucosa. Epithelial cells, macrophages and dendritic cells (DC), are first to encounter the pathogen at mucosal surfaces. Bacteria are equipped of virulence factors enabling chemotaxis and motility, adhesion and /or invasion to mucosal cells, and toxins that poison or subvert the functions of these cells. ***Our consortium will determine the virulence factors and the mechanisms used by the selected pathogens to perform successful mucosal colonization and how this knowledge could be used to develop novel specific therapeutics.***

Brief description of the selected pathogens

Salmonella enterica is a highly diverse group of Gram-negative bacteria, which includes more than 2000 different types (called “serovars”) and it represents one of the leading causes of food-borne infections worldwide. The diseases caused by *S. enterica* range from a self-limiting diarrhoea to a life-threatening systemic infection. The type of disease depends on the *S. enterica* serovar, the host species and the immune status of the host. The molecular factors responsible for the serovar-specific diseases and host range specificities remain largely unclear. In South America, *S. enterica* serovar Enteritidis belongs to the most common causes of food-borne disease. Lack of financial interests by pharmaceutical companies and technical problems (lack of efficiency and safety) have crippled the development of efficient human vaccines for *Salmonella spp*. or therapeutic agents.

Streptococcus pneumoniae or pneumococcus is the main bacterial etiological agent responsible for respiratory tract infections, with 10 million deaths worldwide per year. *S. pneumoniae* causes life-threatening diseases both localized (i.e. otitis media or pneumonia) and invasive (sepsis or meningitis). Infections caused by *S. pneumoniae* are responsible of more than 1 million deaths each year in children less than 5 years of age in the developing world. *S.*

pneumoniae is the most frequent bacterial cause of childhood pneumonia in South America, and the most frequent serotypes are 1, 5, and 14. The commercial 23-valent vaccine based on purified polysaccharide (PS) is not effective among children under 2 or the elderly, which are the main risk groups. Further, the effectiveness against the different serotypes is still a matter of discussion due to the controversial analysis of the clinical trials. The recent introduction of a PS-protein conjugate vaccine has the potential to change this situation, although the vaccine does not include 2 prevalent Latin American serotypes, meaning that this vaccine will have limited protection in this region. Furthermore, this vaccine is difficult to produce and expensive.

Bordetella pertussis causes whooping cough or pertussis in humans, a respiratory disease that is especially severe in young children. The introduction of vaccination with killed whole cell vaccine in 1950 substantially reduced the rates of morbidity and mortality in many regions, inclunding Latin America. In part because of concerns related to the reactogenicity of killed vaccines, acellular vaccines have been developed. These vaccines are formulated in combination with Diphteria-Polio-Tetanus vaccine and injected in young infants. However even though pertussis vaccines are administered to millions of children, recent WHO estimates indicate that pertussis still affects up to 50 million children annually and results in approximately 400,000 deaths. Several countries from Latin America have reported an increased incidence of infection over recent years. Several explanations have been suggested for the re-emergence of pertussis including changes in vaccine potency, decreased vaccine coverage, waning immunity and adaptation of the *B. pertussis* population. The leading hypothesis is based on the antigenic divergence between clinical isolates and vaccine strains. The WHO recommends formulation of vaccines for local use with prevalent local strains.

Clearly there is considerable scope to improve therapies and vaccines against these 3 bacterial pathogens. Our objectives are to explore approaches to early eradicate the bacteria at the mucosal site of infections and to control immunopathology induced by microorganisms.

Mucosal immunity

When constitutive defences are breached, both innate and adaptive mechanisms are stimulated in mucosa in response to microbial signals. The induced innate responses in mucosa aims to block early infection thereby avoiding entry and dissemination of pathogens in deeper tissues. Compared to systemic infections, innate immunity in response to mucosal infections is restricted to mucosa. This concentrates the defences where they are needed and eliminate any undesired systemic effect. Strengthening this inducible innate response is therefore likely to enhance the outcome of mucosal bacterial infection. Epithelial cells, macrophages and DC play a role of sentinel by informing the host of the presence of pathogen. Epithelial cells represent the predominant sentinel cells interacting with luminal environment. In response to microbial stimuli, epithelial cells produce locally anti-microbial agents with broad range of activity, pro-inflammatory cytokines, and chemokines that act directly on microbes and stimulate recruitment and activation of professional phagocytes. ***However, the signals and the specific effector mechanisms used to fight various pathogens in various mucosa are not well characterized. The SavinMucoPath project will improve our knowledge of these mechanisms.***

Adaptive immune responses elicited in peripheral and mucosal tissues differ by the migration of the immune cells. Only lymphocytes stimulated by mucosal infection or vaccination express homing receptors that allow them to return to mucosal sites. The imprinting of a mucosal homing program requires that stimulation occur in mucosa-associated lymphoid tissue. All mucosal surfaces sample and present antigens, but there are differences in the compartmentalization of the immune cells. For instance, nasal immunization triggers responses both in the respiratory and distant mucosal sites and appears the best route for generation pulmonary and genital responses. In contrast, responses following oral immunization are restricted to the intestine and milk during lactation. Mucosal DCs play a crucial role in compartmentalization of immune responses as suggested by the recent findings that intestinal and Peyer's patch's DCs imprint T cells for gut homing. Both epithelial and microbial signals are likely required to promote this compartmentalized response and this issue will be addressed in the present project. This compartmentalized response will be also exploited to develop novel intervention approaches to treat mucosal infections. ***To this aim, we will determine the respective contribution of epithelium and DC using in vivo approaches and in vitro reconstituted cell models, and the immune cross-talk beween gut and lung mucosa in response to infection.***

The innate immune system is a primitive form of non antigen-specific host defence that also helps to shape the adaptive immune responses to antigens derived from microbial pathogens. Activation of innate defence mechanisms is based on recognition of conserved signature molecules of microorganisms referred to as pathogen-associated molecular patterns (PAMP). The detection of PAMPS operates via innate receptors, the PRRs such as the Toll-like receptor (TLR) family. TLRs are transmembrane proteins and act as signal transducers through different signalling pathways, regulating the expression of anti-microbial agents, pro-inflammatory cytokines, and chemokines and promoting the development of adaptive immunity. Thus, upon microbial encounter, the first molecules that trigger pro-inflammatory responses are PAMPs. PAMPs are now considered as new set of drugs with about hundreds of member, which might impact on various human diseases. Each tissue has likely evolved to express the pattern of PRRs required for detection of pathogens that it has to face to. ***Here, we will characterize the natural mechanisms of mucosal immunity against the selected pathogens in order to boost innate and adaptive defences before, during and after infections and in concomitant co-infections with unrelated pathogens. We will then screen novel PAMPs produced by these pathogens that stimulate specifically these mucosal responses. For this purpose, we will develop cell and animal models reporting activation of mucosal immunity.***

Flagellin, the subunit of bacterial flagella, activates various types of cells like DCs, monocytes, intestinal and respiratory epithelial cells in a TLR5 - MyD88 - NF-kappaB-dependent manner. Flagellin triggers a strong epithelial cell response that is characterized by the production of antimicrobial factors and the release of chemokines that attract professional phagocytes (neutrophils and macrophages) and immature DC. Flagellin also activates DC to promote development of T helper 2 (Th2) responses, a feature essential to elicit secretory IgA in mucosa. Thus through PRR, sentinel cells set up the platform for innate mucosal destruction of pathogens and for induction of adaptive immunity by stimulating/attracting DCs. ***In this proposal, we will use Flagellin as a model mucosal PAMP to assess the potency of a bacterial stimulator of immunity in therapeutic and prophylactic mucosal activities and screen other PAMPs from the selected pathogen.***

2.1.2 Contractors involved

Contractor Role*	Contractor n°	Participant n°	Participant name	Contractor short name	Country
CR/CO	1	1	Institut National de la Santé et de la Recherche médicale	INSERM	France
CR**	2	2	Universidad Nacional de la Plata	UNLP	Argentina
CR**	2	3	Universidad Nacional de la Plata	UNLP	Argentina
CR	3	4	Imperial College London	IMPCOL	United Kingdom
CR	4	5	Swiss Federal Institute of Technology (ETH) Zurich	ETHZ	Switzerland
CR	5	6	Universidad de la Republica Oriental del Uruguay	UDELAR	Uruguay
CR	6	7	Pontificia Universidad Catolica de Chile	PUCC	Chile
CR	7	8	Instituto Biologico Argentino	BIOL	Argentina

2.1.3 Main Achievements

1. We have selected isolates specific for Latin American countries including *Streptococcus pneumoniae* serotype 1, *Salmonella enterica* serovar Enteritidis, and *Bordetella pertussis.* These pathogens are locally associated with important rates of morbidity and mortality.
2. We set up or delivered the infection and co-infection models in laboratory animals by mucosal route for all bacterial pathogens (+ influenza virus) described above.
3. We performed the transcriptional analysis of mucosal protective responses to all bacterial pathogens described above.
4. We developed animal and cell models for screening bacterial compounds that induce mucosal immunity (*CCL20-luc, CXCL1-luc,* and *CXCL2-luc*).
5. We dissected the protective mechanisms and pathogenic processes altering innate responses for *Streptococcus* and *Salmonella.*
6. We delivered the proof of concept that a PAMP can be used for therapeutic and prophylactic purposes
7. We further demonstrated that pre-existing challenge can modify the outcome of therapeutic or prophylactic treatments in lungs.
8. We defined new intervention strategies to improve mucosal immunity and dampen mucosal inflammation.

Here follow the most representative findings of the various participants during these 3-years project:

Partner 1 – INSERM – Jean Claude Sirard

- We showed that flagellin is a potent molecule for innate of clearance of mucosal infections.
- We demonstrated that flagellin-mediated innate immunity can operate by 2 pathways. On the luminal side, activation is driven by epithelium. On basal side, activation requires $CD3^{neg}CD4^{+}CD127^{+}$ innate immune cells producing Th17-related cytokines.
- We found that flagellin-mediated mucosal adjuvant effect mainly requires epithelial signaling.
- These findings will be instrumental to develop new therapeutic and vaccinal approaches for mucosal infections.

Partner 2 – UNLP- Martin Rumbo and Daniela Hozbor

- We found that outer membrane vesicles are appropriate candidates for intranasal anti-pertussis vaccine.
- We established the major role of TLR4 signaling in the early protection against pertussis.
- We defined protective innate effectors in pertussis.
- We developed a novel transgenic mice to screen immunomodulatory molecules derived from pathogens.
- We characterized human intestinal mucosa cells and associated lymphoid structures.

Partner 3 – IMPCOL – Tracy Hussell

- We have identified a post-inflammation refractory phenotype in the lung following inflammation that pre-disposes to bacterial super-infection.
- We have further identified a major role for the negative regulator CD200R in innate immune homeostasis in the respiratory tract.
- We identified that blockade of OX40, a T cell co-stimulatory receptor reduce lung inflammatory disease. To prevent infection in the first place, we propose to tailor immune receptors to act as "smart adjuvants" to enhance anti-pathogen immunity.

Partner 4 – ETHZ – Hardt Wolf-Dietrich

- We found that flagellin administration led to a massive reduction of *Salmonella* loads early post infection.
- We observed that protective response against *Salmonella* colitis is mediated by anti-LPS antibodies. This might represent the basis for the future design of a vaccine against *Salmonella* diarrhea.

- We identified a key role of mucosal dendritic cells and Caspase-1 activation in enterocytes in acute *Salmonella* enterocolitis.
- We identified the key pathways and cytokines induced by *Salmonella* in the infected gut mucosa. Their targeting by appropriate drugs may represent a novel strategy to prevent inflammatory colitis.

Partner 5 – UDELAR – Jose Alejandro Chabalgoity

- We delineated a role for Th17 responses in protective immunity against *S. pneumoniae*
- We found that flagellin administration can block an ongoing infection with *S. pneumonia*e
- We demonstrated that it is possible to prepare an effective intranasal whole cell vaccine against the most relevant strains circulating in South America

Partner 6 – PUCC – Alexis Kalergis

- We showed that *S.* Typhimurium impairs antigen presentation by DCs to T cells, due to virulence factors in pathogenicity islands 1 and 2. This process is host specific, and restricted to the DCs derived from host in which Salmonella causes systemic infection.

Partner 7 – BIOL – Patricia Lopez

- We developed a high yield Salmonella flagellin production process. We demonstrated that flagellin is stable at -20, 5, and 25°C when formulated as commercial product. We developed 4 monoclonal antibodies specific for of flagellin.
- We found that bacterial extract used in a pharmaceutical product (INESPECIN) activate innate immunity.
- We produce *B. pertussis* culture in large scale to isolate new antigens.

2.1.4 Problems and corrective actions taken

The delay in the distribution (and effective transfer to contractor accounts) of the budget compared to the effective start date had no major impact on the progresses of the project. Due to exchange losses that will have occurred for the microarray project of the consortium, it was decided that the contractor ETHZ will take in charge the whole project in the name of all participants. This change has not affected the total budget and any deliverables or milestones. For WP that were not fully achieved (mainly for technical reasons), we always proposed a corrective action (See list of deliverables and milestones).

2.2 WORKPACKAGES PROGRESS

Partner 1 – INSERM – Jean Claude Sirard

- We provided partners with flagellin, an immunomodulatory bacterial factor that activate innate and adaptive imunity in mucosa via the TLR innate receptors.
- We studied the mode of action of flagellin in innate and adaptive mucosal responses.
- We coordinated with partners and Dr. Arndt Benecke analysis of mucosal immune response to flagellin, B. pertussis, *S. pneumoniae*, *S. enterica* using microarrays.
- We constructed a transgenic mice (*Ccl20-luc*) to test bacterial molecules sharing immunomodulatory activity of flagellin.
- We managed collaborative activities, follow-up of the WP progress, meetings, courses and reports.

Partner 2 – UNLP - Martin Rumbo and Daniela Hozbor

- We analyzed the innate response controlling *B. pertussis* infection (whooping cough or pertussis)
- We purified and characterized outer membrane vesicles as an acellular vaccine candidate against pertussis.
- We studied the protective effect of flagellin and additional TLR agonists in innate protection against *B. pertussis* infection.

- We investigated the role of *B. pertussis* LPS, not only in the infectious process but also in protection against the disease.
- We generated anti-flagellin monoclonal antibodies.
- We constructed reporter systems with chemokine promoter (*Cxcl1-luc* and *Cxcl2-luc*) and started the generation of transgenic reporter mice line. These animals will be used for screening bacterial molecules with immunomodulatory activity against infections.
- We analyzed human intestinal immune cells and lymphoid structures as a first step of a comparative analysis of human and mice intestinal mucosal immunobiology in collaboration with a transplant center in Buenos Aires.
- We organized the first, second and third year Scientific and Steering Committee of the SavinMucoPath consortium. Besides we organized satellite courses for PhD students and Post-docs on the first and second year.

Partner 3 – IMPCOL – Tracy Hussell

- We analyzed the influence of past infection history on innate and adaptive immunity to respiratory bacteria.
- We identified the late T cell co-stimulators such as OX40 that are responsible for the severity of the first infection. We studied the impact of OX40 blockade on lung inflammatory disease.

Partner 4 –ETHZ –Wolf-Dietrich Hardt

- We analyzed the acute protective and the adaptive immune responses to flagellin using the streptomycin mouse model of *Salmonella* colitis.
- We studied the role of dendritic cells, enterocytes, T-cells and PMN in the colitis.
- We analyzed the transcriptome signatures of gut tissue recovered from mice infected S. *enterica*.
- We constructed a STAT-1-mCherry reporter plasmid, tested it in vitro and generated a transgenic mouse line.
- We managed our projects, the collaborative work and the funding, including help to South American Partners.

Partner 5 – UDELAR – Jose Alejandro Chabalgoity

- We studied the mucosal protective immunity to *S. pneumoniae* as a preamble for the development of new vaccines.
- We set up experimental models of invasive disease and immune protection with clinical isolates of *S. pneumoniae* of the most relevant serotypes in South America.
- We characterized the innate and adaptive protective immune response to *S. pneumoniae* that included a comprehensive set of immunological assays as well as transcriptional profiling by microarray.
- We delineated several intranasal whole cell vaccines and tested them in an attempt to define new vaccines that could be tailored to particular epidemiological situations.

Partner 6 – PUCC – Alexis Kalergis

- We studied the molecular mechanisms used by *Salmonella* to interfere with the activation of the adaptive immune response.

Partner 7 – BIOL – Patricia Lopez

- We developed protocols to cultivate *B. pertussis* and *S. enterica* for large scale production of *B. pertussis* outer membrane vesicles (partner 2) and *Salmonella* flagellin.
- We studied the stability of flagellin and evaluated its potential development as a product.
- We investigated the presence of immunomodulators in a bacterial extract used in the manufacture of a pharmaceutical product licensed by BIOL (INESPECIN).

% tasks initially planned and achieved	Partner 1 INSERM	Partner 2 UNLP	Partner 3 IMPCOL	Partner 4 ETHZ	Partner 5 UDELAR	Partner 6 PUCC	Partner 7 BIOL	Total partners
Workpackage 1	100	100		100	100			100
Workpackage 2				100				100
Workpackage 3					90			90
Workpackage 4		100						100
Workpackage 5	100	100		100	100			100
Workpackage 6	25	100					70	73
Workpackage 7		50						50
Workpackage 8	50	50						50
Workpackage 9				20				20
Workpackage 10			100					100
Workpackage 11			100					100
Workpackage 12					60			60
Workpackage 13						100		100
Workpackage 14	80	50						63
Workpackage 15	100	100	100	100	100	100	100	100
% per participant	**76**	**82**	**100**	**70**	**75**	**100**	**71**	**>80**

For WP that were not fully achieved according to initial plans, corrective actions have been conducted as described in Lists of Deliverables and Milestones.

2.2.1 List of deliverables

Deliverable N°	Deliverable Title	Date Due	Nature	Dissemi-nation level	Delivery (YES or NO)	Actual/Fore cast delivery date	Correcting Action (Cancel or New Action)	Estimated indicative person-months	Used indicative person-months	Lead contractor
1.1	Flagellin and control preparations. Procedures and doses for mucosal (nasal and oral) activation	3	P	CO	YES	16/02/2007 3/11/2008 06/03/2009	Several preparations of Flagellin have been performed for the progress of the work	1,8	6,3	**1**
1.2	Potency of Flagellin as therapeutic agent against mucosal infections	12	P	CO	YES	30/9/2008	study of the activation of innate response using different TLR ligands at the moment of the infection	2,0	2,8	**2**
1.2	Potency of Flagellin as therapeutic agent against mucosal infections	12	P	CO	YES	30/9/2009		5,0	8,7	**3**
1.2	Potency of Flagellin as therapeutic agent against mucosal infections	12	P	CO	YES	1/9/2009		5,0	15,0	**5**
1.2	Potency of Flagellin as therapeutic agent against mucosal infections	12	P	CO	YES	31/12/2007 30/09/2009	Experiments have been conducted to confirm results obtained in 2007 to achieve publication	6,0	12,5	**6**
1.3	Potency of Flagellin as mucosal adjuvants against mucosal infections	24	P	CO	YES	30/9/2009		1,0	1,0	**2**
2.1	Cryo-embedded gut tissues from wild type mice infected with wild type S. Enteritidis and wild type S. Typhimurium for WP5.	12	P	CO	YES	1/3/2007	shifted to RNA extraction from total cecum tissue	3,0	3,0	**5**
2.2	Cryo-embedded gut tissues from infected MyD88-/-, TRIF-/-, TLR2-/-, TLR4-/- and wild type mice. Also material from infections with site specific Salmonella mutants available from WP5.	18	P	CO	YES	1/6/2009	additional resources were added using the remaining resources from WP9 (corrective action)	22,0	35,7	**5**
2.3	Cryo-embedded gut tissues from Salmonella infected MyD88-/- - wild type bone marrow chimaeric mice and from mice with no/defective DC or depleted NK1.1 cells for WP5.	24	P	CO	YES	1/3/2009		18,0	10,0	**5**
2.4	Report on the contribution of innate signalling and the type of cells initiating Salmonella enterocolitis	36	R	CO	YES	30/9/2009		11,5	6,1	**5**
3.1	Delivery of clinical isolates of S. pneumoniae and Salmonella enterica serovar Enteritidis of main importance in Latin America for WP2 and 13.	1	P	CO	YES (partially)	1/9/2007		1,0	1,0	**6**
3.2	Pneumonia and nasopharyngeal infection models set up on resistant and susceptible mice to S. pneumoniae.	6	P	CO	YES	1/6/2007		9,0	9,0	**6**
3.3	Delivery of lung RNA from early infections (4 and 24h) with S. pneumoniae of resistant and susceptible animals for micro-array experiments (WP5).	6	P	CO	YES	1/9/2007		5,0	5,0	**6**
3.4	Table of time course of chemokine and activation pathways elicited on the different mice strains by the selected clinical relevant isolates.	12	R	CO	YES	30/09/2008 30/09/2009	Additional analyses have been performed in 2009	12,0	20,0	**6**
3.5	Immune correlate of natural resistance vs. susceptibility to clinical isolates.	18	R	CO	YES	30/9/2009		9,0	15,0	**6**
4.1	Delivery of lung RNA from asymptomatic and productive mouse infections with B. pertussis at early time points (4 and 24h) for micro-array experiments (WP5).	4	P	CO	YES	31/7/2007		2,0	2,0	**3**
4.2	Production of OMV from B. pertussis to be evaluated as protective antigen	6	P	CO	YES	31/08/2007 30/09/2009	Preparation of OMV has been continued in 2009	2,0	6,0	**3**

4.3	Purification of B. pertussis Prn to be evaluated as protective antigen.	10	P	CO	YES	30/3/2009	We have changed the strategy. Instead of purifying Prn from B. pertussis, we cloned the gene but we could not express the protein. We used this strategy to obtain fim3 and PTxS1 and we succeeded	6,0	12,0	**3**
4.4	Purification of B. pertussis FHA to be evaluated as protective antigen.	15	P	CO	YES	30/7/2008		10,0	10,0	**3**
4.5	Purification of B. pertussis PT to be evaluated as a protective antigen	19	P	CO	YES	30/8/2008		11,0	10,5	**3**
4.6	Report of results from comparative analysis of protection using different combination of PAMPs/antigens.	30	R	CO	YES	1/4/2009		14,0	5,0	**3**
4.7	Report on protective capacity of selected treatment against clinical Bordetella isolates.	36	R	CO	YES	20/7/2009		15,5	8,8	**3**
5.1	Pattern of PRR expression in mouse and human respiratory and intestinal tract.	16	R	CO	NO	Cancelled	New action: functional characterization of innate response receptors in human mucosal cell organized lymphoid structures and diffuse lymphoid tissue	3,0	4,0	**2**
5.2	Plasmids expressing RNAi specific for the innate genes.	20	P	CO	NO	Cancelled	Analysis of effectors of protective response by neutralizing antibodies	6,0	0,0	**1**
5.3	Characterization of gene expression by real-time PCR and topography by laser dissection microscopy – identification of 2 genes coding innate effectors with potential role in pathogen clearance in mucosa	20	P	CO	YES	1/9/2009	Instead micro-dissection, we have done chimera mice to define contribution of radio-resistant versus radio-sensitive cells.	2,0	1,6	**1**
5.4	Characterization of RNAi plasmids on cell models.	22	R	CO	YES	30/9/2009	Cancelled. New action: identification of TLR4 dependent pathways critical for control of B. pertussis infection	2,0	16,0	**2**
5.5	Delivery of 1 or 2 transgenic lines for silencing candidate innate effectors by RNAi expression.	28	P	CO	NO	Cancelled	Analysis of effectors of protective response by neutralizing antibodies	4,0	0,0	**1**
5.5	Delivery of 1 or 2 transgenic lines for silencing candidate innate effectors by RNAi expression.	28	P	CO	NO	Cancelled	Analysis of effectors of protective response by neutralizing antibodies	4,0	0,0	**2**
5.6	Completion of micro-array analysis and delivery of gene expression pattern specific for three pathogens – Hypothesis on protective innate effectors	30	P	CO	YES	30/9/2009		9,0	21,4	**1**
5.6	Completion of micro-array analysis and delivery of gene expression pattern specific for three pathogens – Hypothesis on protective innate effectors	30	P	CO	YES	30/9/2009		11,0	12,0	**2**
5.6	Completion of micro-array analysis and delivery of gene expression pattern specific for three pathogens – Hypothesis on protective innate effectors	30	P	CO	YES	30/9/2009		4,0	8,4	**3**
5.6	Completion of micro-array analysis and delivery of gene expression pattern specific for three pathogens – Hypothesis on protective innate effectors	30	P	CO	YES	30/9/2009		4,0	7,0	**5**
5.6	Completion of micro-array analysis and delivery of gene expression pattern specific for three pathogens – Hypothesis on protective innate effectors	30	P	CO	YES	30/9/2009		6,0	15,6	**6**
6.1	Delivery of the different cleared bacterial lysates/bacterial pellet before lysis.	6	P	CO	YES	1/4/2007		3,0	2,2	**8**
6.2	Delivery of mucosa-specific PAMP(s) derived from S. pneumoniae.	16	P	CO	NO	Cancelled	S. pneumoniae bullk culture in Participant 8 facilities was canceled due to delay in progress with the other pathogens	6,0	0,0	**8**

6.3	Delivery of mucosa-specific PAMP(s) derived from S. enterica.	18	P	CO	YES	1/6/2008	Flagellin and fractions from lysates of Salmonella enterica	3,0	2,0	**2**
6.3	Delivery of mucosa-specific PAMP(s) derived from S. enterica.	18	P	CO	YES	1/6/2008	Flagellin and fractions from lysates of Salmonella enterica	5,0	4,2	**1**
6.3	Delivery of mucosa-specific PAMP(s) derived from S. enterica.	18	P	CO	YES	1/4/2008		6,0	10,0	**8**
6.4	Delivery of mucosa-specific PAMP(s) derived from B. pertussis.	20	P	CO	YES	30/9/2009	preparation of membrane fractions from B. pertussis	3,0	8,6	**2**
6.4	Delivery of mucosa-specific PAMP(s) derived from B. pertussis.	20	P	CO	YES	1/8/2008	preparation of membrane fractions from B. pertussis and purified immunogens	6,0	3,0	**3**
6.4	Delivery of mucosa-specific PAMP(s) derived from B. pertussis.	20	P	CO	YES	01/08/2008 30/9/2009	Additional preparation were performed in 2009	14,5	31,3	**8**
6.5	Delivery of results from the stability tests.	34	R	CO	YES	30/9/2009		12,0	3,0	**8**
6.6	Delivery results from the analysis of scaling and cost evaluation	36	R	CO	YES	30/9/2009		5,0	3,0	**8**
7.1	Delivery of Caco-2-ccl20-luc and T84-ccl20-luc reporter cells	6	P	CO	YES	28/2/2007		1,0	1,0	**2**
7.2	Delivery of cxcl2-(luc-gfp-lacZ) reporter plasmids (mouse and human promoters).	9	P	CO	YES	31/5/2007		2,0	3,0	**2**
7.3	Delivery of A549-ccl20-luc and m-ICcl2-ccl20-luc reporter cells.	9	P	CO	YES (partially)	30/9/2009	selection of suitable cell line and stable transfectants selection protocol	4,0	6,0	**2**
7.4	Characterisation of the initial reporter constructions received from WP9.	9	R	CO	NO		transference of the material cancelled due to inappropriate functionality	2,0	0,0	**2**
7.5	Delivery of cxcl2-(luc-gfp-lacZ) reporter cells (Caco-2 and A549). Delivery of h-bd2/m-bd3 reporter constructions.	12	P	CO	YES	28/2/2008	New action: CXCL1-luceferase construct characterized	5,0	8,7	**2**
7.6	Characterisation of the transcription factor-specific reporter constructs from WP9.	15	R	CO	NO		Abandonned due to problem with transgenic constructs	2,0	0,0	**2**
7.7	Delivery of h-bd2/m-bd3 reporter cell lines.	18	P	CO	YES (partially)	30/9/2009	CXCL-1 luciferase stable cell line preparation in progress	2,0	6,0	**2**
8.1	Delivery of NF-kappaB-luc transgenic mice and mouse expression plasmid ccl20-luc.	1	P	CO	YES	1/11/2006		0,0	0,0	**1**
8.2	Delivery of mouse transgenic for ccl20-luc.	6	P	CO	YES	1/4/2009		8,5	4,3	**1**
8.3	Delivery of plasmid encoding a novel fusion between an innate (pro-inflammatory) gene promoter and luc.	12	P	CO	NO	Cancelled	Analysis of effectors of protective response by neutralizing antibodies against cytokines and innate cells.	3,0	0,0	**1**
8.3	Delivery of plasmid encoding a novel fusion between an innate (pro-inflammatory) gene promoter and luc.	12	P	CO	YES	30/9/2009	CXCL1- and CXCL2-luciferase constructs	3,0	4,0	**2**
8.4	First PAMPs screened in vitro on ccl20-luc reporter cells.	12	R	CO	NO	Abandonned	Investigation is still in progress	6,0	0,0	**1**
8.4	First PAMPs screened in vitro on ccl20-luc reporter cells.	12	R	CO	YES	31/8/2007	Has been used to define the efficacy of flagellins prepared by participant 8	3,0	5,3	**2**
8.5	First PAMPs screened in vivo for the ability to stimulate mucosal immunity (ccl20-luc).	20	R	CO	YES	30/7/2008	in vivo effects assessed by RT-qPCR	2,5	2,7	**2**
8.6	Real-time imaging of mouse transgenic for ccl20-luc and standardized procedures for imaging and luciferase assays in respiratory and intestinal mucosa.	20	R	CO	YES	1/4/2009	Standardized assays for luciferase activity ex vivo.	10,0	0,5	**1**
8.7	Delivery of second line of transgenic mouse.	22	P	CO	YES (partially)	30/9/2009	Construction of transgenic mice for CXCL1-Luc and CXCL2-Luc are still in progress	10,0	10,2	**2**
8.8	Second set of PAMPs screened in vivo for the ability to stimulate mucosal immunity (second transgenic mouse line).	36	R	CO	NO	Abandonned	Action cancelled due to delay in access to transgenic mice.	8,0	0,0	**2**
9.1	Streptomycin-pre-treated mouse model for Salmonella enterocolitis; training and advice for other WPs.	1	P	CO	YES	1/10/2006		1,0	1,0	**5**
9.2	Delivery of pNFkB-mHoneydew, pAP-1-tdTomato constructs for generation of transgenic animals and to other WPs for further in	12	P	CO	YES	30/9/2007	developed STAT-1 constructs ; these have been delivered by month 12	8,0	8,0	**5**

	vitro testing.									
9.3	Delivery of mice transgenic for pNFkB-mHoneydew, pAP-1-tdTomato.	24	P	CO	NO	Abandonned	Attempts to generate STAT1-mCherry transgenic mice were not conclusive after several injection and screening. This WP was cancelled in End of 2008; remaining resources were used for WP1 (mucosal vaccination project) and WP5	26,0	20,1	**5**
9.4	Established performance of the pNFkB-mHoneydew and pAP-1-tdTomato reporter mice for inflammation studies. Time and cell type resolved map of pro-inflammatory signalling during S. Typhimurium and S. Enteritidis enterocolitis.	36	R	CO	NO	abandonned	cancelled in End of 2008; remaining resources were used for WP1 (mucosal vaccination project) and WP5	5,0	0,0	**5**
10.1	Mouse model of S. pneumoniae and H. Influenzae infection.	12	P	CO	YES	1/9/2007		3,0	3,0	**4**
10.2	Mouse model of concurrent lung/lung infection.	24	P	CO	YES	30/9/2008		20,0	19,5	**4**
10.3	Mouse model of concurrent gut/lung or lung/gut infection.	36	P	CO	YES	1/7/2009		19,5	11,1	**4**
11.1	Delivery of Influenza and RSV mouse models: monitoring of pathogen clearance, analysis of pathology and immune responses.	1	P	CO	YES	1/11/2006		0,0	0,0	**4**
11.2	Delivery of OX-40 ligand fusion proteins, agonist and antagonist CD200 receptor antibodies, CD200 fusion proteins.	1	P	CO	YES	1/11/2006		0,0	0,0	**4**
11.3	Mechanisms of innate imprinting and effect of T cell-APC modulation	36	R	CO	YES	30/9/2009		43,0	33,2	**4**
12.1	Results on protection efficacy of the different formulations.	24	R	CO	YES	30/9/2009		18,5	12,0	**6**
12.2	Chart of immunological mechanisms involved in the response elicited different formulations.	30	R	CO	YES	30/9/2009		20,0	7,0	**6**
12.3	Chart of comparative analysis of response in naturally resistant strains vs. protective vaccinated strains after challenge.	36	R	CO	YES	30/9/2009	The new action was to study the innate and adaptive immune response in the model of protection.	20,0	7,0	**6**
13.1	*Salmonella strains (clinical isolates) expressing Ovalbumin as neo-antigen. Set up of DC cultures and infection with Salmonella and T cell activation assays.*	3	P	CO	YES	31/12/2006		9,7	9,7	**7**
13.2	T cell activation assays by Salmonella infected DCs.	6	P	CO	YES	1/4/2007		9,7	9,7	**7**
13.3	Set up of adoptive transfer experiments using bacteria-specific transgenic T cells.	12	P	CO	YES	1/10/2007		9,7	9,7	**7**
13.4	*Salmonella mutants, targeting virulence genes.*	18	P	CO	YES	1/4/2008		13,0	12,7	**7**
13.5	Characterization of phenotype of Salmonella mutants and their capacity to interfere with DC function.	24	R	CO	YES	1/10/2008		14,0	14,0	**7**
13.6	Completion of experiments using immune complexes containing virulent Salmonella and targeting to Fc receptors on DCs.	30	R	CO	YES	1/4/2009		24,5	21,9	**7**
13.7	Completion of experiments to evaluate the participation of DCs on the dissemination of virulent Salmonella in mice.	36	R	CO	YES	30/9/2009		22,9	9,4	**7**
14.1	Primary epithelial cell cultures optimized.	18	P	CO	YES	30/6/2008		3,5	4,1	**2**
14.2	Chart with time course effects on gene expression of different epithelial cells with Flagellin and different PAMPs.	24	R	CO	YES	30/9/2008	flagellin effects assessed on intestinal and epithelial cell lines at whole genome scale. Selected markers were assessed for other TLR stimuli on same cell lines	2,5	2,2	**2**

14.2	Chart with time course effects on gene expression of different epithelial cells with Flagellin and different PAMPs.	24	R	CO	YES	30/9/2008	flagellin effects assessed on bronchial epithelial cell lines.	6,0	0,7	**1**
14.3	Isolation techniques of mucosal DC from different mucosal tissues for co-culture experiments	24	P	CO	YES	30/9/2008		2,5	4,5	**2**
14.3	Isolation techniques of mucosal DC from different mucosal tissues for co-culture experiments	24	P	CO	NO	Abandonned		6,0	0,0	**1**
14.4	Chart with microscopy, gene expression and protein expression of different co-culture systems upon stimulation with selected PAMPs	30	R	CO	YES	30/9/2009	Analysis was done only in vitro using chimera mice	6,0	5,7	**1**
14.4	Chart with microscopy, gene expression and protein expression of different co-culture systems upon stimulation with selected PAMPs	30	R	CO	YES	30/9/2009	Original deliverable cancelled. New action: characterization of lymphoid structures and cell populations in human intestinal mucosa	16,0	14,4	**2**
15.01	Minutes of the kick-off meeting. Delivery of procedures for reporting activities, and describe the mobilization of resources and budget. Explanation of mode of action of the consortium. Final agreement of the function of the Steering Committee and the decision-making procedures concerning the scientific aspects of the project.	1	R	CO	YES	18/12/2006		1,0	1,0	**1**
15.02	First Payment of contractors (80% months 1-18)	1	P	CO	YES	4/12/2006		0,0	0,0	**1**
15.03	Short scientific report and Time sheets for personnel of Months 1-3	3	R	CO	NO	Canceled	Decision to perform every 4 months :2 short reports and 1 annual report per period	0,2	0,0	**1**
15.04	Functional SavinMucoPath Website	3	P	CO	YES	10/12/2006		0,5	0,5	**1**
15.05	Short scientific report and Time sheets for personnel of Months 4-6	6	R	CO	YES	28/2/2007		0,5	0,5	**1**
15.06	Minutes from conference call with Contractors – Mid-annual interactions to solve on-going questions of the Consortium.	6	R	CO	NO	Canceled		0,1	0,0	**1**
15.07	Short scientific report and Time sheets for personnel of Months 7-9	9	R	CO	NO	Canceled	Decision to perform every 4 months :2 short reports and 1 annual report per period	0,2	0,0	**1**
15.08	Minutes of 1st year Steering Committee and symposium in South America	12	R	CO	YES	13/11/2007		0,5	0,4	**1**
15.08	Minutes of 1st year Steering Committee and symposium in South America	12	R	CO	YES	13/11/2007		0,2	0,4	**2**
15.09	1st annual scientific, management Report, and financial statement from each contractors	12	R	CO	YES	13/11/2007		0,5	1,5	**1**
15.09	1st annual scientific, management Report, and financial statement from each contractors	12	R	CO	YES	13/11/2007		0,5	1,5	**2**
15.1	Short scientific report and Time sheets for personnel of Months 13-15	15	R	CO	YES		decision to perform each 4 months was taken during First Year PSC Meeting	0,1	0,1	**2**
15.1	Short scientific report and Time sheets for personnel of Months 13-15	15	R	CO	YES		decision to perform each 4 months was taken during First Year PSC Meeting	0,2	0,3	**1**
15.11	Short scientific report and Time sheets for personnel of Months 16-18	18	R	CO	NO	canceled		0,1	0,0	**2**
15.11	Short scientific report and Time sheets for personnel of Months 16-18	18	R	CO	NO	canceled		0,1	0,0	**1**
15.12	Minutes from conference call with Contractors – Mid-2nd year interactions to solve on-going questions of the Consortium.	18	R	CO	YES	1/4/2008		0,2	0,5	**1**
15.12	Minutes from conference call with Contractors – Mid-2nd year interactions to solve on-going questions of the Consortium.	18	R	CO	YES	1/4/2008		0,1	0,1	**2**
15.13	Second Payment of contractors (80% months 12-30)	18	P	CO	YES	12/7/2008	Delay in payment and bank transfer	0,1	0,1	**1**

15.14	Short scientific report and Time sheets for personnel of Months 19-21	21	R	CO	NO		decision to perform each 4 months was taken during First Year PSC Meeting	0,1	0,0	**1**
15.14	Short scientific report and Time sheets for personnel of Months 19-21	21	R	CO	NO		decision to perform each 4 months was taken during First Year PSC Meeting	0,1	0,0	**2**
15.15	Minutes of 2nd year Steering Committee in South America	24	R	CO	YES	1/10/2008		0,2	0,5	**1**
15.15	Minutes of 2nd year Steering Committee in South America	24	R	CO	YES	1/10/2008		0,2	0,1	**2**
15.16	2nd annual scientific Report, financial statement and Audit Certificates from each contractors	24	R	CO	YES	1/10/2008	No audit requested for the second period	0,5	2,5	**1**
15.16	2nd annual scientific Report, financial statement and Audit Certificates from each contractors	24	R	CO	YES	1/10/2008	No audit requested for the second period	0,5	1,5	**2**
15.17	Short scientific report and Time sheets for personnel of Months 25-27	27	R	CO	YES	1/4/2009		0,5	0,5	**1**
15.18	Minutes from conference call with Contractors – Mid-3rd year interactions to solve on-going questions of the Consortium	30	R	CO	YES	From 1/10/2008 to 30/9/2009	Conference call were done every 2-weeks with Martin Rumbo and every 1-2 Months with other contractors	0,1	0,1	**1**
15.19	Third Payment of contractors (80% months 30-36)	30	P	CO	YES	15/7/2009		0,1	0,1	**1**
15.2	Minutes of 3rd year Steering Committee in Europe	35	R	CO	YES	30/9/2009	We have done a compendium sent to the scientific officer	0,5	0,5	**1**
15.21	3rd annual scientific Report, financial statement and Audit Certificates from each contractors	36	R	CO	YES	15/12/2009		2,0	2,0	**1**
15.21	3rd annual scientific Report, financial statement and Audit Certificates from each contractors	36	R	CO	YES	15/12/2009		1,0	0,9	**2**
15.22	Final dissemination plan and exploitation plan	36	R	CO	YES	15/12/2009		0,2	0,2	**1**
15.22	Final dissemination plan and exploitation plan	36	R	CO	YES	15/12/2009		0,5	0,5	**2**
15.23	Last payment	36	P	CO	pending	in 2010		0,0	0,0	**1**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	3,7	**3**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	7,8	**4**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	1,7	**5**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	2,6	**6**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	10,4	**7**
15.24	Management of scientific activity and financial issues, reporting, preparation and participation to steering committee	36	P	CO	YES		This deliverable takes in account the management activity of all partners that were not initially included for this activity	1,5	5,7	**8**

2.2.2 List of Milestones

Table 2: Milestones List

List all milestones, giving date of achievement and any proposed revision to plans.

Milestone no.	Milestone name	Date due Month	Delivery (YES or NO)	Actual/Fore cast delivery date	Correcting Action (Cancel or New Action)	Lead Contractor
1,1	Production of Flagellin for downstream applications	1 - 3	YES	16/2/2007		1
1,2	Protective effect of Flagellin as therapeutic agents for ongoing respiratory infections with *S. pneumoniae*	3 - 12	YES	1/5/2009		6
1,3	Protective effect of Flagellin as therapeutic agents for ongoing respiratory infections with *B. pertussis*	3 - 12	YES	30/9/2009	We performed experiment in the context of the phenomenon termed Stimulated Innate Resistance	3
1,4	Protective effect of Flagellin as therapeutic agents for ongoing intestinal infections with *S.* Enteritidis	3 - 12	YES	1/5/2009	We analyzed innate protection and also protection by adaptive immune responses (corrective action)	5
1,5	Characterization of Flagellin potency as mucosal adjuvants for pathogenic bacteria antigens – Correlation between the mucosal responses (IgA and CD4) induced by mucosal immunization and the protective efficacy against subsequent infections against pathogens	9 - 24	YES	1/5/2009		3
2,1	Comparison of the virulence of *S.* Enteritidis clinical isolates from South America with the well-characterized laboratory strain *S.* Typhimurium SL1344.	0 - 12	YES	30/9/2008		5
2,2	Role of MyD88 and TRIF in *Salmonella* enterocolitis. Due to redundancy, TLR2 and TLR4 may not yield overt differences in disease (histopathology) but are expected to yield subtle differences in pro-inflammatory or defensive gene expression as detected by gene chip analysis (WP5).	9 - 18	YES	1/1/2009		5
2,3	Completion of bone marrow chimaeras study. Definition of the respective role of epithelium/stromal cells vs. leukocytes/lymphocytes in innate signalling and enterocolitis.	12 - 24	YES	1/4/2009		5
2,4	Completion of S. Enteritidis/ Typhimurium comparison. Similarities and differences in the requirement of innate signalling, DC and NK cells.	18 - 36	YES	30/9/2009	based on publication (Suar et al., 2009), we shifted this work to S. typhimurium (same mechnaism, but better tool available)	5
3,1	Set up of nasopharyngeal and pneumonia infection model using relevant clinical isolates of *S. pneumoniae* on three different mice strains.	1 - 6	YES	1/6/2007		6
3,2	Physiological characterization of the innate response in the different mouse infection models.	6 - 12	YES	31/3/2008		6
3,3	Full characterization of the adaptive response in the different infection models. We expect to find differences between the resistant and susceptible strains that may be exploited for improving vaccination strategies.	6 - 18	NO	Cancelled		6
4,1	Set up of intranasal infection model with *Bordetella pertussis*	1 - 4	YES	30/12/2006		3
4,2	Production of *B. pertussis* antigens and set up of mouse infection model for innate immunity analysis.	1 - 19	YES	31/8/2007		3
4,3	Characterization of protective capacity of *B. pertussis* OMV delivered via the nasal route employing the murine respiratory infection model.	6 - 12	YES	31/8/2007		3

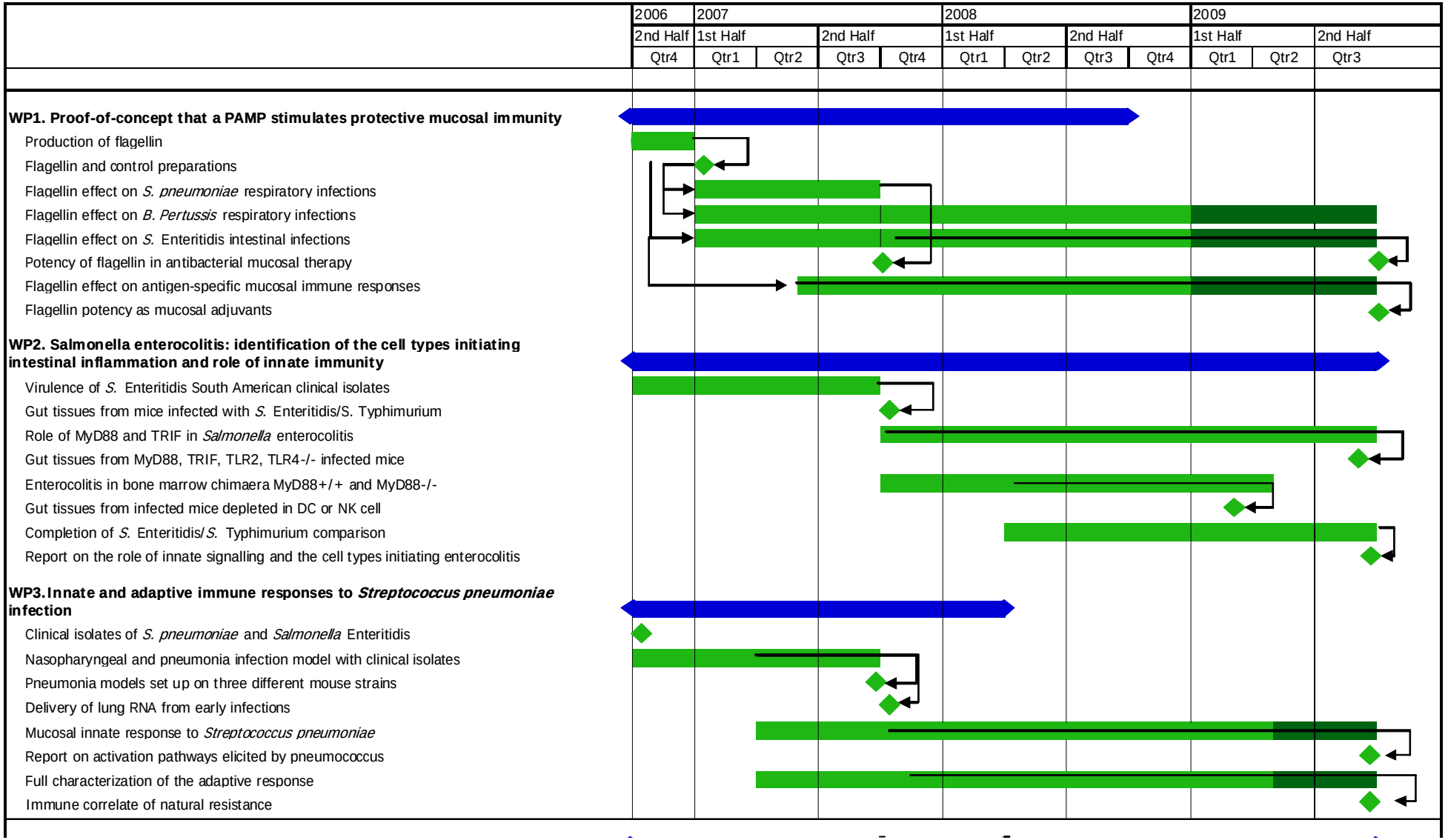
4,4	Characterization of protective capacity of *B. pertussis* Prn delivered via the nasal route employing the murine respiratory infection model.	10 - 18	YES	20/2/2009		3
4,5	Characterization of protective capacity of *B. pertussis* FHA and PT delivered via the nasal route employing the murine respiratory infection model.	15 - 25	YES	30/9/2009	we changed to fimbria because their poteintial role as PAMPs. We finished with the purification procedures. We are currently analysing their capability to induce innate immune response.	3
4,6	Characterization of protective capacity of *B. pertussis* antigens in different combination delivered via the nasal route employing the murine respiratory infection model. Report of results from comparative analysis of protection using different PAMPs as mucosal adjuvants.	15 - 30	YES	30/9/2009		3
4,7	Comparative analysis of protective capacity/immune response of vaccines delivered via nasal route and intra-peritoneal route.	24 31	YES	30/9/2009		3
4,8	Most efficient treatment to protect against pertussis infection	28 - 36	YES	30/9/2009		3
5,1	Analysis of micro-arrays to define pattern associated to innate protection against mucosal infections (Bordetella start month 4, *Streptococcus* start month 6, *Salmonella* start month 12, 18 and 24). Data mining and statistics will be performed in the laboratory of partner 1 and 2.	8 - 30	YES	30/6/2009		1
5,1	Analysis of micro-arrays to define pattern associated to innate protection against mucosal infections (Bordetella start month 4, *Streptococcus* start month 6, *Salmonella* start month 12, 18 and 24). Data mining and statistics will be performed in the laboratory of partner 1 and 2.	8 - 30	YES	30/6/2009		3
5,1	Analysis of micro-arrays to define pattern associated to innate protection against mucosal infections (Bordetella start month 4, *Streptococcus* start month 6, *Salmonella* start month 12, 18 and 24). Data mining and statistics will be performed in the laboratory of partner 1 and 2.	8 - 30	YES	30/6/2009		5
5,1	Analysis of micro-arrays to define pattern associated to innate protection against mucosal infections (Bordetella start month 4, *Streptococcus* start month 6, *Salmonella* start month 12, 18 and 24). Data mining and statistics will be performed in the laboratory of partner 1 and 2.	8 - 30	YES	30/6/2009		6
5,2	Activation of NF-kappaB-luc fusion upon mucosal infection.	9 - 16	YES	30/9/2009	Most analysis were performed using qRTPCR.	1
5,2	Activation of NF-kappaB-luc fusion upon mucosal infection.	9 - 16	NO		we have just received a GFP-NFKB reporter mouse which we are going to study after the end of this INCO project.	5
5,3	Analysis of PRR expression in mouse and human respiratory and intestinal samples.	12 - 16	NO	30/9/2009	This issue has been canceled due to new findings in the litterature.	2
5,4	Micro-array validation by RT-PCR – Topography of expression using laser dissection microscopy.	16 - 20	NO		pending on setting up of LDM facility in Argentina	2
5,5	Generation of plasmids for RNAi of innate genes.	19 - 20	YES	1/3/2009	RNAi stragegy was abandoned upon Steering Commettee discussion in Sept 08. Antibody depletion of different populations was choosed instead. Participant 1 has subcontracting the production of a panel of depleting antibodies	1
5,6	Validation of RNAi of innate genes on cell culture.	20 - 22	YES	30/9/2009	RNAi stragegy was abandoned upon Steering Commettee discussion in Sept 08. Antibody depletion of different populations was choosed instead. Participant 1 has subcontracting the production of a panel of depleting antibodies	2
5,7	Construction of mice transgenic for RNAi expression **(sub-contracting)**.	22 - 28	NO		RNAi stragegy was abandoned upon Steering Commettee discussion in Sept 08. Antibody depletion of different populations was choosed instead. Participant 1 has subcontracting the production of a panel of depleting antibodies	1
5,8	Comparison of innate responses to various pathogens.	28 - 35	YES	30/9/2009		1

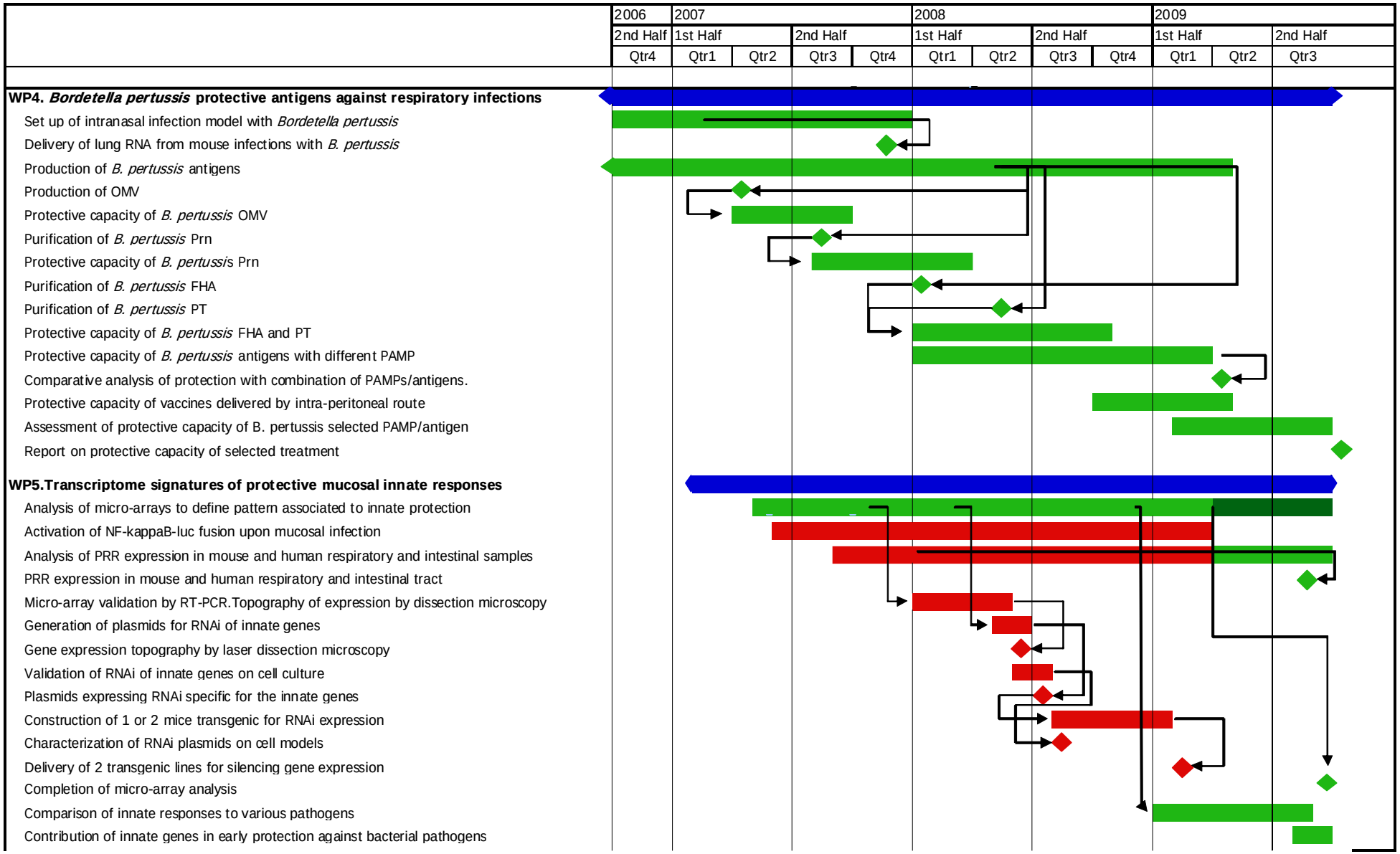
5,8	Comparison of innate responses to various pathogens.	28 - 35	YES	30/9/2009		2
5,8	Comparison of innate responses to various pathogens.	28 - 35	YES	30/9/2009		5
5,8	Comparison of innate responses to various pathogens.	28 - 35	YES	30/9/2009		6
5,9	Proof-of-concept of contribution of innate genes in early protection against bacterial pathogens.	34 - 36	YES	30/9/2009		1
6,1	Selected strains are transferred from partners 3 and 6. Total of 10 strains of *B. pertussis*, *S. pneumoniae* and *S. enterica* serovars.	1 -	YES	1/6/2007		3
6,2	Bacterial cultures, death kinetics and controls established.	0 - 2	YES	1/4/2007		8
6,3	Production of bacterial lysates completed.	2 - 6	YES	1/8/2007		8
6,4	Purification and identification of PAMPs from the selected pathogens that activate mucosa and epithelial cells.	4 - 20	NO			1
6,4	Purification and identification of PAMPs from the selected pathogens that activate mucosa and epithelial cells.	4 - 20	YES (partially)	30/9/2009	Salmonella and Bordetella fractions in progress	2
6,5	Analysis of stability/toxicity of isolated PAMP molecules. We expect to determine the stability of active compounds after various treatments (constant temperature vs. freeze/thaw cycles) on reporter cells delivered by partner 2 and WP7.	20 - 34	YES	30/9/2009	Flagellin stability study conducted in collaboration with Participant 8	2
6,5	Analysis of stability/toxicity of isolated PAMP molecules. We expect to determine the stability of active compounds after various treatments (constant temperature vs. freeze/thaw cycles) on reporter cells delivered by partner 2 and WP7.	20 - 34	YES	30/9/2009	Flagellin stability study conducted in collaboration with Participant 2	8
6,6	Scaling up feasibility and cost evaluation completed. This analysis will be critical to evaluate the possibilities of transfer of technology and define the putative industrial partners that can be targeted as part of the dissemination and exploitation plans.	34 - 36	YES	30/9/2009		8
7,1	Cloning and Characterization of mice and human *cxcl2* promoter.	1 - 3	YES	february-07		2
7,2	Transfection and selection of stable clones of A549 and m-ICcl2 reporter cells containing *ccl20-luc*.	1 - 9	NO			2
7,3	Preparation of *cxcl2* reporter constructions.	3 - 9	YES	1/5/2007		2
7,4	Report of results from comparative analysis of potency of the different bacterial preparations from WP6 on A549-*ccl20-luc* reporter cells and Caco-2-*ccl20-luc* reporter cells.	6 - 9	YES (partially)	July-07	This milestone was mainly achieved with the test of flagellin preparation	2
7,5	Selection of stable cell transfectants for reporting *cxcl2* expression. Completion of other reporter constructions.	9 - 12	YES	30/9/2009	Report constructs (CXCL1-luc and CXCL2- luc) finished	2
7,6	Completion of stable transfectants with other reporter plasmids.	15 - 18	NO			
8,01	Construction of the *ccl20-luc* transgenic mice **(sub-contracting)**.	1 - 6	YES	1/4/2009		1
8,1	First results of *in vivo* PAMP screening with second line of transgenic animals	28 - 36	NO		Transgenic mice generation in progress	
8,02	Identification of bacteria/PAMPs that stimulate *in vitro ccl20-luc* reporter epithelial cells.	6 - 12	YES (partially)	30/9/2009	This milestone was mainly achieved with the test of flagellin preparation	1
8,2	Identification of bacteria/PAMPs that stimulate *in vitro ccl20-luc* reporter epithelial cells.	6 - 12	YES (partially)	July-07	fractions from WP6 screened	2
8,3	Construction and *in vitro* characterization of the reporter fusion for generation of a second transgenic mice.	8 - 12	YES	August 07		2
8,4	Characterization of fidelity of *ccl20-luc* mouse for monitoring epithelial pro-inflammatory responses (expression in steady-state and stimulated conditions and topography).	6 - 12	YES	1/4/2009		1
8,5	*In vivo* characterization of reporter fusion for generation of a second transgenic mice.	12 - 14	YES	31/12/2008		2
8,6	Construction of the second transgenic mice that report stimulation of mucosal innate immunity **(sub-contracting).**	14 - 22	YES (partially)	30/9/2009	Subcontracting started in March09, still in progress	2
8,7	*In vivo* PAMP screening with *ccl20-luc* animals.	15 - 20	NO			1

8,8	Characterization of fidelity of the second transgenic mouse line for monitoring mucosal pro-inflammatory responses.	22 - 28	NO		Transgenic mouse line screening in progress	2
8,9	Characterization of fidelity of the third transgenic mouse line for monitoring mucosal pro-inflammatory responses.	28 - 36	NO		First candidates screened in September09	2
9,1	Generation and in vitro testing of pNFkB-mHoneydew, pAP-1-tdTomato, pIRF3-mCherry, pSTAT1-mPlum constructs.	1 - 6	NO	changed to new STAT-1 mCherry construct	we constructed and delivered a STAT-1 construct, instead	5
9,2	Characterization of reporter shuttle vectors for pNFkB-mHoneydew, pAP-1-tdTomato (= DNA constructs for generation of respective transgenic mice) and for pIRF3-mCherry, pSTAT1-mPlum.	6 - 12	NO	changed to new STAT-1 mCherry construct	we constructed and delivered a STAT-1 construct, instead	5
9,3	Construction of pNFkB-mHoneydew, pAP-1-tdTomato reporter mice for advanced analysis of S. Enteritidis and S. Typhimurium infections.	12 - 24	NO	changed to new STAT-1 mCherry construct	mouse construction failed; shifted the remaining resourced of WP9 to WP1 (vaccination model for Salmonella diarrhea) and WP5 (gene expression analysis) by the end of 2008	5
9,4	Analysis of pro-inflammatory signalling during S. Typhimurium and S. Enteritidis enterocolitis.	24 - 36	YES	30/9/2009		5
10,1	establishment of *S. pneumoniae* and *H. Influenzae* infection model.	1 - 12	YES	1/9/2007		4
10,2	Characterization of Influenza/*S. pneumoniae* co-infection model.	12 - 24	YES	1/9/2008		4
10,3	Establishment of the Reovirus infection model. Characterization of RSV/*S. pneumoniae* co-infection model.	12 - 24	YES	1/9/09		4
10,4	Characterization of Influenza/*H. Influenzae* co-infection model. Effect of reovirus gut infection on Influenza lung infection and vice-versa.	24 - 36	YES	06-July-2009		4
11,1	Effect on innate imprinting by TLR ligands on Influenza infection. Immunogenicity and efficacy of OX40- and CD200R-related reagents as adjuvants for inactivated Influenza virus.	1 - 12	YES	1/9/2007		4
11,2	Similar experiments are extended to the RSV model.	12 - 24	YES	10/7/2009		4
11,3	Mechanisms of innate imprinting. Effect of CD200 receptor ligation on reovirus infection.	5 - 36	YES	1/6/2009		4
12,1	Protection studies using the different intranasal formulations of *S. pneumoniae* vaccines.	12 - 24	YES	30/9/2008		6
12,2	Analysis of the immunological response against different formulated vaccines.	15 - 30	YES	30/4/2009		6
12,3	Evaluation of changes in lung response in vaccination vs. vaccination and challenge situation. Comparison of the response pattern elicited in a protective vaccination vs. response in naturally resistant mice strains. We expect to determine the different mechanisms to prevention of infection in the natural resistant strains vs. vaccinated protected strains to propose rational strategies to improve human vaccination.	18 - 36	YES partially	30/9/2009	we did not manage to find a resistant mouse strain. So we did all the evaluation in protected vs non-protected animals	6
13,1	Construction of recombinant *Salmonella.*	1 - 3	YES	31/12/2006		7
13,2	Based on our preliminary studies we hypothesize that virulent *Salmonella* impairs T cell activation by preventing processing and presentation of bacterial antigens by DCs.	1 - 6	YES	31/3/2007		7
13,3	Virulent *Salmonella* would be able to survive inside DCs.	6 - 12	YES	30/9/2007		7
13,4	Construction of *Salmonella* mutants.	6 - 18	YES	31/3/2008		7
13,5	The identification of *Salmonella* genes responsible for interfering with DC function will suggest molecular mechanisms for the ability of this pathogen to escape adaptive immunity. In addition, these new virulence factors could be considered novel targets for antibiotic treatment and the strains of *S.* Typhimurium attenuated by mutations on those genes could be used as vaccines.	18 - 24	YES	30/9/2008		7
13,6	Targeting *Salmonella* to FcRs on DCs will enhance antigen processing and presentation to T cells. Thus, the mechanism developed by this pathogen to evade DC function could be hindered. In addition, delivering antigens to FcRs on DCs could work as a novel vaccination strategy to promote T cell immunity against intracellular bacterial pathogens.	12 - 30	YES	30/4/2009		7

13,7	Considering the potential capacity of virulent *Salmonella* to interfere with DC function, we expect that these cells be used as carriers for systemic dissemination of bacteria.	12 - 36	YES	30/9/2009		7
14,1	Setting up of primary epithelial cell cultures from intestinal and lung origin	9 - 18	YES (partially)	30/9/2009	Primary epitelial culture established	2
14,2	Results on gene expression of different epithelial models upon microbial product stimulation. Comparative analysis of response considering microbial niche (airways vs. intestinal pathogens) and epithelial cell type will indicate the differences in the innate response signalling against similar PAMPs elicited in epithelial cells from different origin. This would have an impact on our understanding on host-pathogen interactions and on the immune response elicited by PAMPs according to the mucosal delivery route.	18 - 24	YES (partially)	30/9/2009	performed on a restricted set of markers. Will amplify the profiling in next period	2
14,3	Setting up of different co-culture systems. Isolation techniques of different cell types from human intestinal tissues standardized.	18 - 24	YES (partially)	30/9/2009	Human intestinal cell populations isolation and characterization achieved	2
14,4	Microscopy analysis of co-cultures. Differences on topology of DC-epithelium interaction in lung and intestinal models may reflect the sampling capacity of different mucosa. Impact of microbial product on this dynamic process is anticipated to be also dependent on the mucosal environment. These results will be important for deciding targeting strategies of vaccination according to the mucosal route employed.	24 - 30	NO		Cancelled. Co-culture systems were not developed.	2
14,5	Gene expression results on the co-culture system obtained. These results will reflect the impact of the epithelial-immune cell interaction on gene expression.	30 - 36	NO		Cancelled. Co-culture systems were not developed.	2
14,6	Comparative analysis of epithelial vs. co-culture system gene expression validated. These results will tend to identify the contribution of individual cell types to the mucosal response. Differences between the different mucosal tissues are anticipated.	30 - 36	NO		Cancelled. Co-culture systems were not developed.	2
15,1	Organization of the kick-off meeting in Europe. Presentation of procedures for managing and reporting activities, description of the the mobilization of resources and budget. Description of projects.	1 -	YES	31/10/2006		1
15,2	Kick-off meeting to recall the scientific, legal, financial and ethical procedures within our project. Informations from the coordinator to partners	1 -	YES	31/10/2006		1
15,3	Organization of the 1st Steering Committee and mid-term symposium in South America by the Co-coordinator and organization of the mid-term symposium.	12 -	YES	20/8/2007		1
15,4	Organization of the 2nd Steering Committee by the Project Manager, the Coordinator and the Co-ordinator in South America.	24 -	YES	20/9/2008		1
15,5	Organization of the 3rd Steering Committee in Europe by the Coordinator team. Preparation of the dissemination and exploitation plans.	35 -	YES	31/8/2009		1

2.2.3 Project time tables and status

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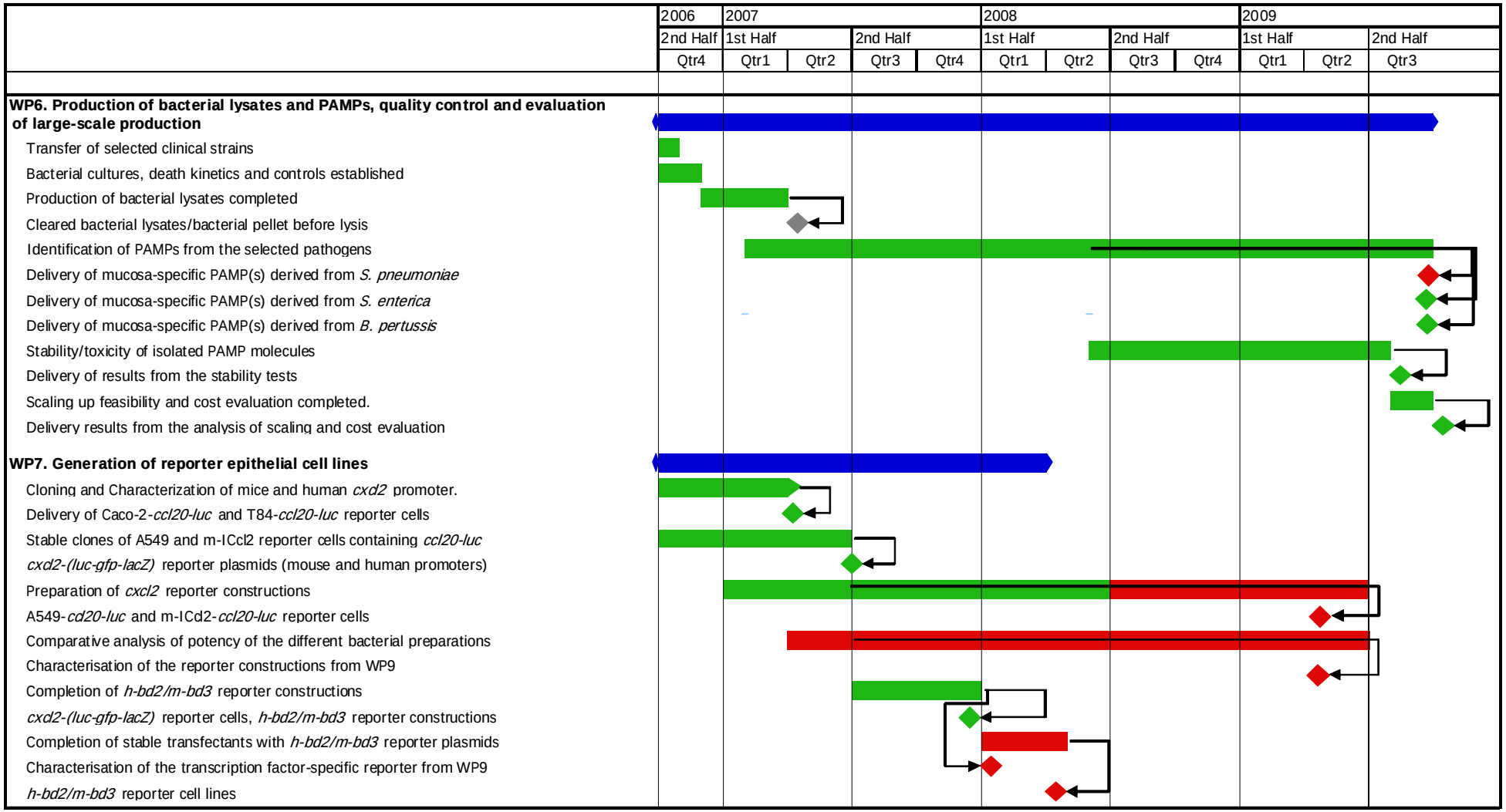
WP4. Bordetella pertussis protective antigens against respiratory infections
Set up of intranasal infection model with Bordetella pertussis
Delivery of lung RNA from mouse infections with B. pertussis
Production of B. pertussis antigens
Production of OMV
Protective capacity of B. pertussis OMV
Purification of B. pertussis Prn
Protective capacity of B. pertussis Prn
Purification of B. pertussis FHA
Purification of B. pertussis PT
Protective capacity of B. pertussis FHA and PT
Protective capacity of B. pertussis antigens with different PAMP
Comparative analysis of protection with combination of PAMPs/antigens.
Protective capacity of vaccines delivered by intra-peritoneal route
Assessment of protective capacity of B. pertussis selected PAMP/antigen
Report on protective capacity of selected treatment
WP5.Transcriptome signatures of protective mucosal innate responses
Analysis of micro-arrays to define pattern associated to innate protection
Activation of NF-kappaB-luc fusion upon mucosal infection
Analysis of PRR expression in mouse and human respiratory and intestinal samples
PRR expression in mouse and human respiratory and intestinal tract
Micro-array validation by RT-PCR.Topography of expression by dissection microscopy
Generation of plasmids for RNAi of innate genes
Gene expression topography by laser dissection microscopy
Validation of RNAi of innate genes on cell culture
Plasmids expressing RNAi specific for the innate genes
Construction of 1 or 2 mice transgenic for RNAi expression
Characterization of RNAi plasmids on cell models
Delivery of 2 transgenic lines for silencing gene expression
Completion of micro-array analysis
Comparison of innate responses to various pathogens
Contribution of innate genes in early protection against bacterial pathogens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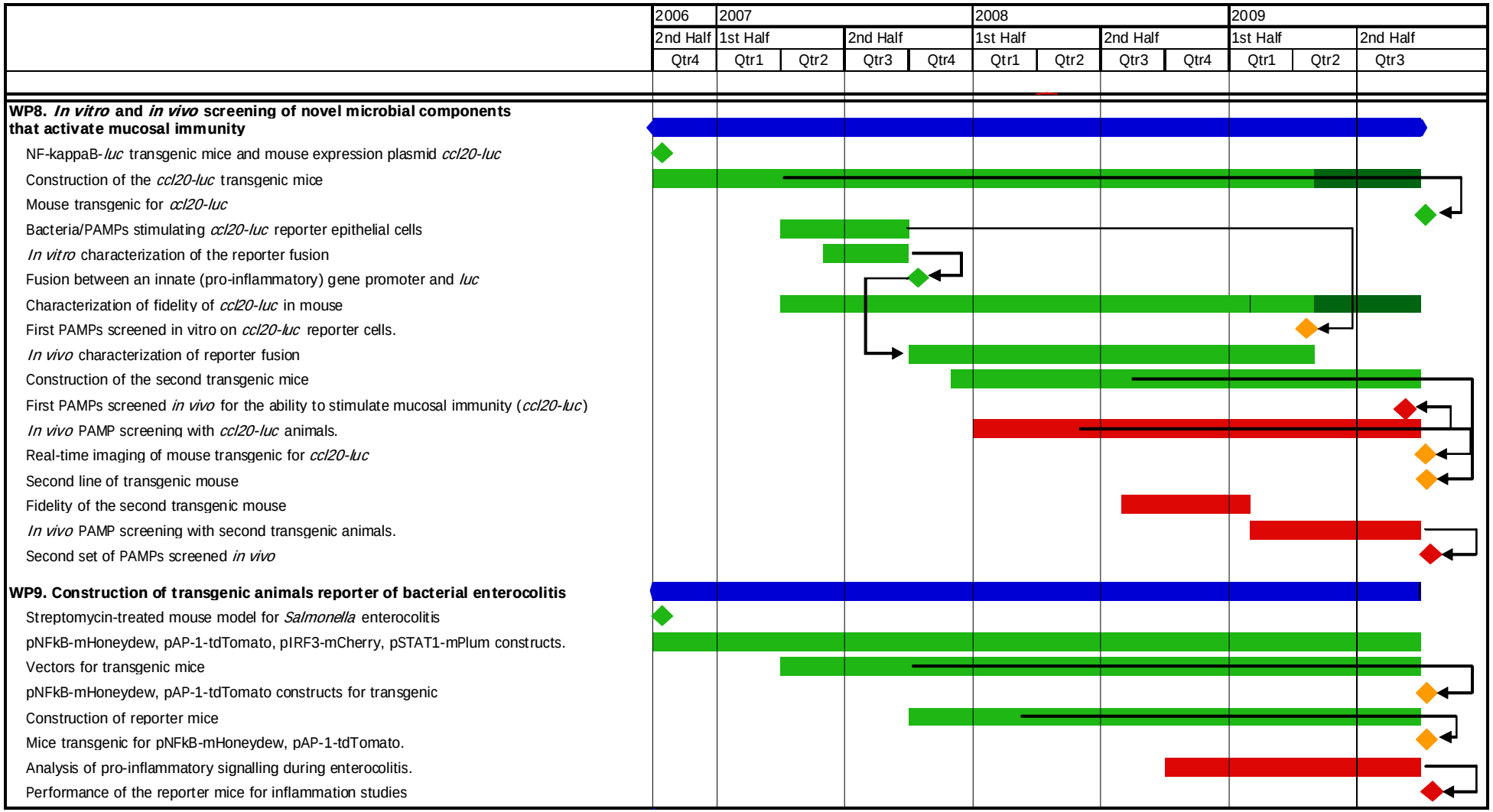
WP6. Production of bacterial lysates and PAMPs, quality control and evaluation of large-scale production
Transfer of selected clinical strains
Bacterial cultures, death kinetics and controls established
Production of bacterial lysates completed
Cleared bacterial lysates/bacterial pellet before lysis
Identification of PAMPs from the selected pathogens
Delivery of mucosa-specific PAMP(s) derived from S. pneumoniae
Delivery of mucosa-specific PAMP(s) derived from S. enterica
Delivery of mucosa-specific PAMP(s) derived from B. pertussis
Stability/toxicity of isolated PAMP molecules
Delivery of results from the stability tests
Scaling up feasibility and cost evaluation completed.
Delivery results from the analysis of scaling and cost evaluation
WP7. Generation of reporter epithelial cell lines
Cloning and Characterization of mice and human cxd2 promoter.
Delivery of Caco-2-ccl20-luc and T84-ccl20-luc reporter cells
Stable clones of A549 and m-ICcl2 reporter cells containing ccl20-luc
cxd2-(luc-gfp-lacZ) reporter plasmids (mouse and human promoters)
Preparation of cxcl2 reporter constructions
A549-cd20-luc and m-ICd2-ccl20-luc reporter cells
Comparative analysis of potency of the different bacterial preparations
Characterisation of the reporter constructions from WP9
Completion of h-bd2/m-bd3 reporter constructions
cxd2-(luc-gfp-lacZ) reporter cells, h-bd2/m-bd3 reporter constructions
Completion of stable transfectants with h-bd2/m-bd3 reporter plasmids
Characterisation of the transcription factor-specific reporter from WP9
h-bd2/m-bd3 reporter cell lines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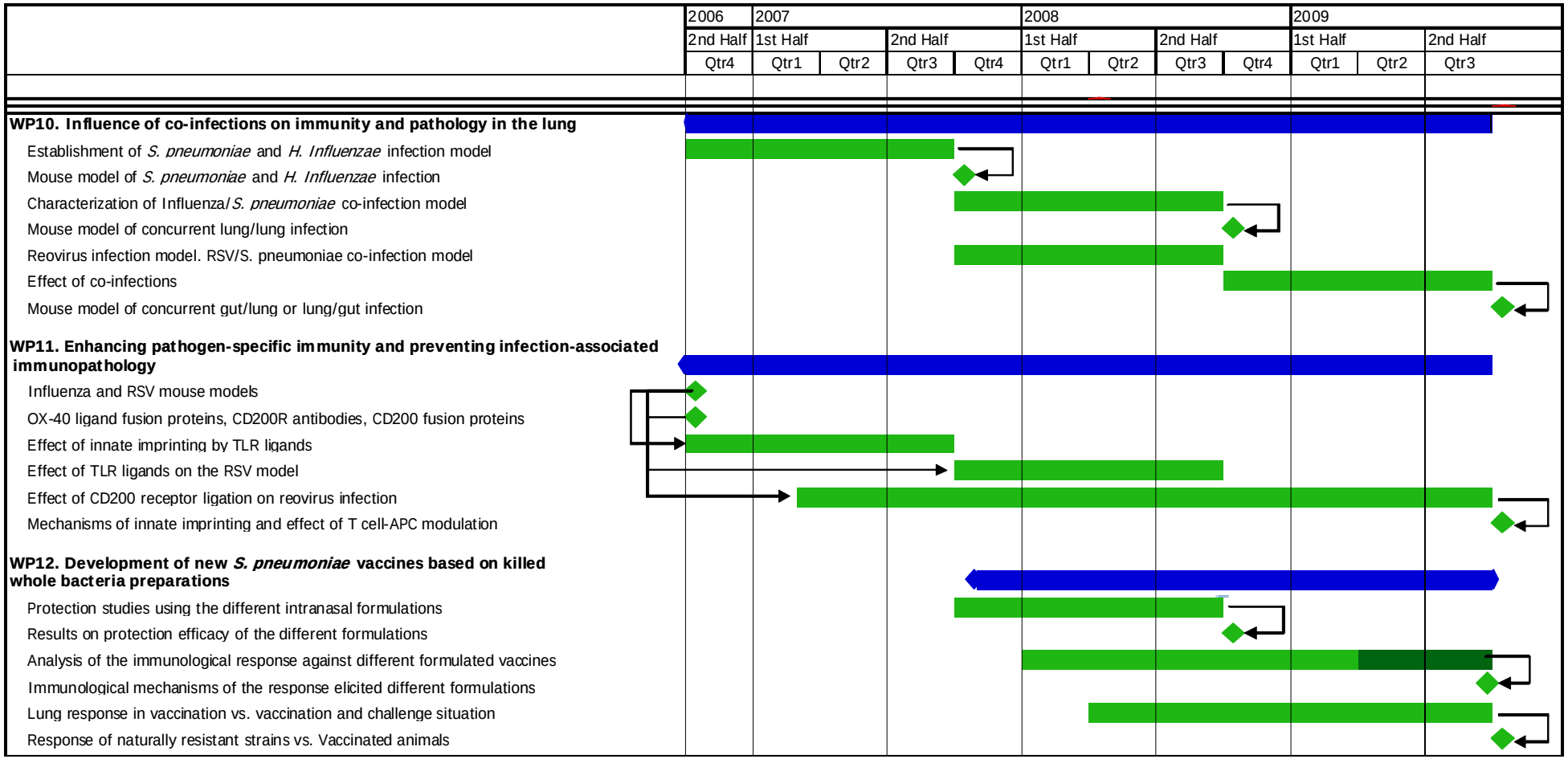
WP8. In vitro and in vivo screening of novel microbial components that activate mucosal immunity
NF-kappaB-luc transgenic mice and mouse expression plasmid ccl20-luc
Construction of the ccl20-luc transgenic mice
Mouse transgenic for ccl20-luc
Bacteria/PAMPs stimulating ccl20-luc reporter epithelial cells
In vitro characterization of the reporter fusion
Fusion between an innate (pro-inflammatory) gene promoter and luc
Characterization of fidelity of ccl20-luc in mouse
First PAMPs screened in vitro on ccl20-luc reporter cells.
In vivo characterization of reporter fusion
Construction of the second transgenic mice
First PAMPs screened in vivo for the ability to stimulate mucosal immunity (ccl20-luc)
In vivo PAMP screening with ccl20-luc animals.
Real-time imaging of mouse transgenic for ccl20-luc
Second line of transgenic mouse
Fidelity of the second transgenic mouse
In vivo PAMP screening with second transgenic animals.
Second set of PAMPs screened in vivo
WP9. Construction of transgenic animals reporter of bacterial enterocolitis
Streptomycin-treated mouse model for Salmonella enterocolitis
pNFkB-mHoneydew, pAP-1-tdTomato, pIRF3-mCherry, pSTAT1-mPlum constructs.
Vectors for transgenic mice
pNFkB-mHoneydew, pAP-1-tdTomato constructs for transgenic
Construction of reporter mice
Mice transgenic for pNFkB-mHoneydew, pAP-1-tdTomato.
Analysis of pro-inflammatory signalling during enterocolitis.
Performance of the reporter mice for inflammation studies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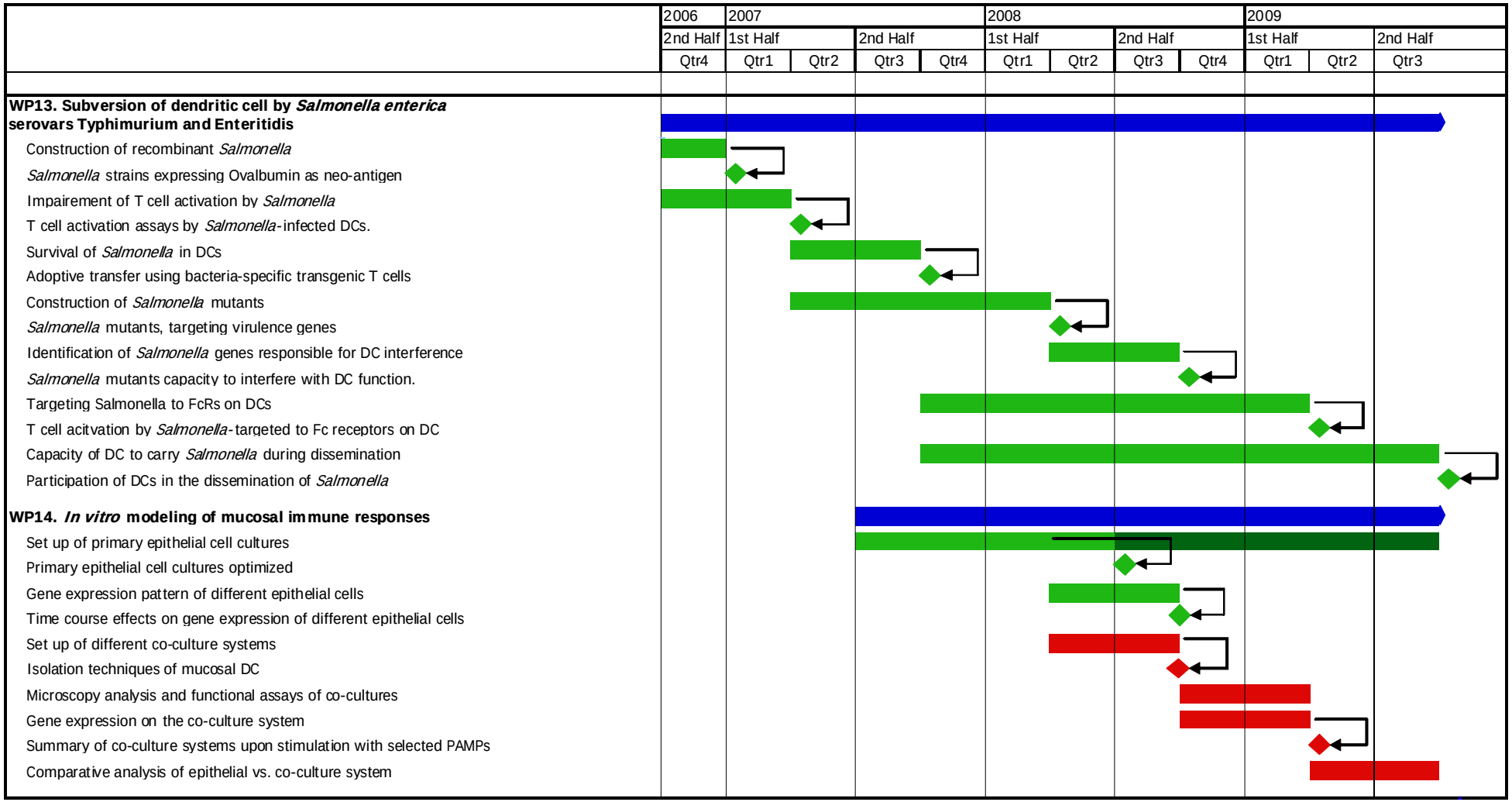
WP10. Influence of co-infections on immunity and pathology in the lung
Establishment of S. pneumoniae and H. Influenzae infection model
Mouse model of S. pneumoniae and H. Influenzae infection
Characterization of Influenza/S. pneumoniae co-infection model
Mouse model of concurrent lung/lung infection
Reovirus infection model. RSV/S. pneumoniae co-infection model
Effect of co-infections
Mouse model of concurrent gut/lung or lung/gut infection
WP11. Enhancing pathogen-specific immunity and preventing infection-associated immunopathology
Influenza and RSV mouse models
OX-40 ligand fusion proteins, CD200R antibodies, CD200 fusion proteins
Effect of innate imprinting by TLR ligands
Effect of TLR ligands on the RSV model
Effect of CD200 receptor ligation on reovirus infection
Mechanisms of innate imprinting and effect of T cell-APC modulation
WP12. Development of new S. pneumoniae vaccines based on killed whole bacteria preparations
Protection studies using the different intranasal formulations
Results on protection efficacy of the different formulations
Analysis of the immunological response against different formulated vaccines
Immunological mechanisms of the response elicited different formulations
Lung response in vaccination vs. vaccination and challenge situation
Response of naturally resistant strains vs. Vaccinated animals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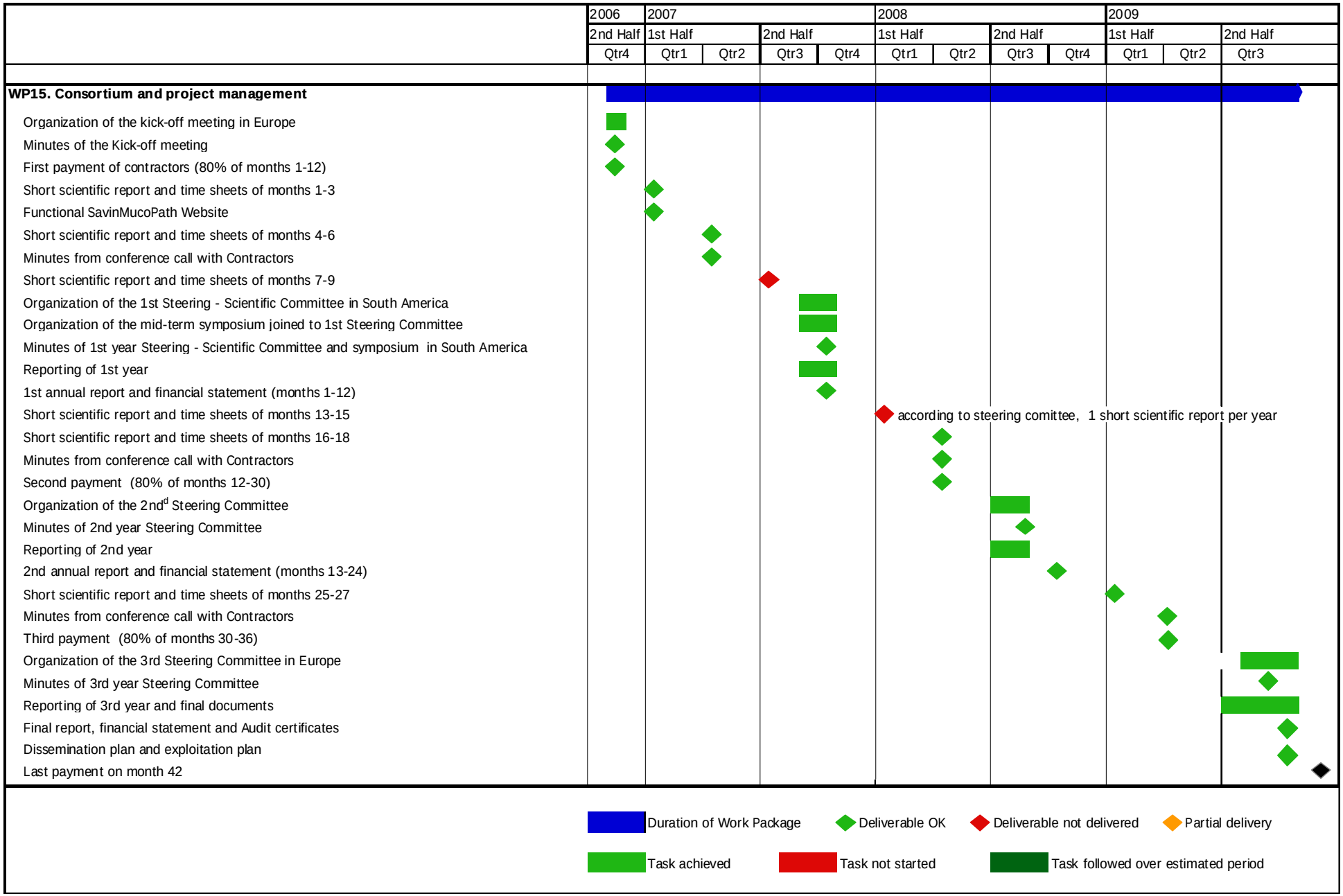
WP13. Subversion of dendritic cell by Salmonella enterica serovars Typhimurium and Enteritidis
Construction of recombinant Salmonella
Salmonella strains expressing Ovalbumin as neo-antigen
Impairement of T cell activation by Salmonella
T cell activation assays by Salmonella-infected DCs.
Survival of Salmonella in DCs
Adoptive transfer using bacteria-specific transgenic T cells
Construction of Salmonella mutants
Salmonella mutants, targeting virulence genes
Identification of Salmonella genes responsible for DC interference
Salmonella mutants capacity to interfere with DC function.
Targeting Salmonella to FcRs on DCs
T cell acitvation by Salmonella-targeted to Fc receptors on DC
Capacity of DC to carry Salmonella during dissemination
Participation of DCs in the dissemination of Salmonella
WP14. In vitro modeling of mucosal immune responses
Set up of primary epithelial cell cultures
Primary epithelial cell cultures optimized
Gene expression pattern of different epithelial cells
Time course effects on gene expression of different epithelial cells
Set up of different co-culture systems
Isolation techniques of mucosal DC
Microscopy analysis and functional assays of co-cultures
Gene expression on the co-culture system
Summary of co-culture systems upon stimulation with selected PAMPs
Comparative analysis of epithelial vs. co-culture system


2006
2007
2008
2009
2nd Half
1st Half
2nd Half
1st Half
2nd Half
1st Half
2nd Half
Qtr4
Qtr1
Qtr2
Qtr3
Qtr4
Qtr1
Qtr2
Qtr3
Qtr4
Qtr1
Qtr2
Qtr3
WP15. Consortium and project management
Organization of the kick-off meeting in Europe
Minutes of the Kick-off meeting
First payment of contractors (80% of months 1-12)
Short scientific report and time sheets of months 1-3
Functional SavinMucoPath Website
Short scientific report and time sheets of months 4-6
Minutes from conference call with Contractors
Short scientific report and time sheets of months 7-9
Organization of the 1st Steering - Scientific Committee in South America
Organization of the mid-term symposium joined to 1st Steering Committee
Minutes of 1st year Steering - Scientific Committee and symposium in South America
Reporting of 1st year
1st annual report and financial statement (months 1-12)
Short scientific report and time sheets of months 13-15
according to steering comittee, 1 short scientific report per year
Short scientific report and time sheets of months 16-18
Minutes from conference call with Contractors
Second payment (80% of months 12-30)
Organization of the 2nd Steering Committee
Minutes of 2nd year Steering Committee
Reporting of 2nd year
2nd annual report and financial statement (months 13-24)
Short scientific report and time sheets of months 25-27
Minutes from conference call with Contractors
Third payment (80% of months 30-36)
Organization of the 3rd Steering Committee in Europe
Minutes of 3rd year Steering Committee
Reporting of 3rd year and final documents
Final report, financial statement and Audit certificates
Dissemination plan and exploitation plan
Last payment on month 42
Duration of Work Package
Deliverable OK
Deliverable not delivered
Partial delivery
Task achieved
Task not started
Task followed over estimated period